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Dorsomedial Thalamotomy (VIII): Changes of Blood Components.

By

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In order to investigate the influences of dorsomedial thalamotomy[1] upon blood components, this research has been undertaken. In this paper, the results on red cells, leucocytes, lymphocytes and blood sedimentation rate are reported in comparison with the results corresponding after lobotomy.

The investigations were performed in preoperative (one week before) and postoperative (one, two and three weeks and 30 days after) stadiums. The results are shown in Table I. Those 13 cases consisted of 6 thalamotomized cases, 4 lobotomized ones, and 3 thalamotomized ones, in which lobotomy had been made 3 or more months ago. Here it must

TABLE I.

Results of Measurement in 13 Cases of Schizophrenia.

Examination	Date	Thalamotomized cases						L. & T. C.			Lobot. cases			
		1	2	3	4	5	6	7	8	9	10	11	12	13
Number of red cells (in million)	before oper.	4.5	4.8	4.5	4.8	4.9	5.1	4.8	5.5	4.0	5.8	4.1	4.5	3.8
	7 d. after	3.7	4.2	4.2	3.6	4.6	3.6	4.0	4.0	4.0	...	...	...	...
	14 d. "	4.4	4.3	4.0	4.5	4.9	**5.3**	4.5	4.2	**4.9**	4.4	**4.5**	**4.6**	**4.8**
	30 d. "	3.2	...	...	4.1	4.4	...	4.7	...	**4.1**	...	...	...	...
Number of leucocytes (in thousand)	before oper.	6.1	5.2	6.9	4.8	6.2	7.2	7.6	5.6	8.3	7.2	7.4	7.7	6.8
	7 d. after	5.7	4.7	4.5	4.9	4.7	5.1	4.4	**5.9**	5.7	...	...	...	...
	14 d. "	5.6	4.4	5.6	4.1	5.6	4.5	6.4	**5.9**	4.6	**8.8**	**8.3**	**7.9**	**7.2**
	30 d. "	5.3	...	...	4.5	**7.1**	...	3.6	...	4.2	...	...	...	...
Lymphocytes (in %-value)	before oper.	29	27	19	31	23	48	35	29	40	36	21	36	53
	7 d. after	27	**38**	22	**38**	**25**	29	30	**31**	**35**	...	...	...	...
	14 d. "	**34**	**34**	**40**	16	**24**	36	**39**	**34**	32	**40**	5	30	28
	30 d. "	27	...	...	31	**27**	...	**42**	...	**39**	...	...	...	...
Blood sediment. rate (in aver. val.)	before oper.	12	25	6	2	10	2	3	10	15	6	17	7	10
	7 d. after	**26**	**52**	**20**	**3**	**12**	**3**	2	**16**	**23**	...	...	...	...
	14 d. "	**19**	**56**	**9**	**11**	**20**	2	2	**12**	**30**	6	11	**19**	10
	30 d. "	**31**	...	...	...	**12**	...	2	...	**20**	...	...	...	...

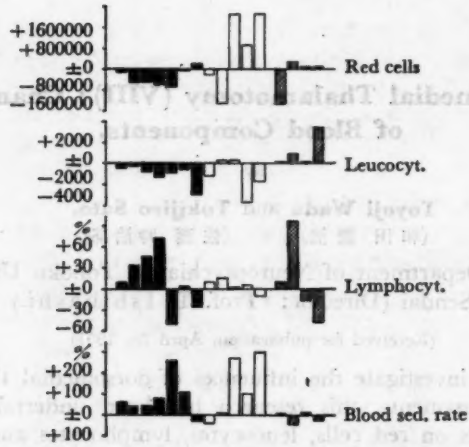

Fig. 1. Alternation of the changes of blood components in 16 cases of schizophrenia in the first two weeks after operation, in comparison with the values in the preoperative stadium. Black squares indicate the data of thalamotomized cases; white squares, those of thalamotomized and lobotomized cases, and oblique asqures those of lobotomized cases.

be noticed that those results are given by no other means having influences upon blood components, than the operation, since we have omitted the cases complaining of somatic disorders, such as tuberculosis, ascariasis, remarkable anemia, heart diseases, etc., and also, those treated with sulfamide-preparation or penicillin.

The results might be summarized as follows: erythropenia, lymphocytosis, leucopenia and increase of blood sedimentation rate (b. s. r.) occured almost uniformly in the first one week after thalamotomy operation. In the first 2 and 3 weeks, the tendency toward such changes were still observed (s. Fig. 1). After 39 days, the changes seemed almost to return to preoperative level. The tendency of changes in cases of prefrontal lobotomy alone was opposite; erythrocytosis, leucocytosis, lymphocytosis and irregular pictures in b. s. r. In cases of lobotomy with thalamotomy, the results were manifest, although the tendency toward lymphocytosis and increase of b. s. r. were recognized.

As above mentioned, the changes following thalamotomy operation were reversible, which means that the changes are rather transient. Consequently, it may be said that thalamotomy does not bring forth any such severe disturbances in blood components, that do not allow its clinical application.

It seems difficult to make adequate comment on such changes. Sporadically, there were exceptional cases. One of them might result

from the abnormality of blood components of schizophrenia themselves: in fact, there were certain abnormal data in preoperative stadium. On other hands, dorsomedial thalamotomy means an invasion to the dorsomedial thalamic part; however, the funcution of the dorsomedial thalamic nucleus for blood components seems still obscure. If the changes described above actually resulted from the operation itself, it may be unable to deny the function of dorsomedial uncleus[2] in blood components regulation. Whether or not this owes to the indirect mechnism, such as a transient disturbance of hypothalamic part due to interruption of internuclear connections,[3][4] must be anew pursued by experimental researches.

Conclusion.

The changes were uniform: erythropenia, leucopenia, lymphocytosis and increase of blood sedimentation rate. However, those changes were almost transient and seemed to tend generally to return to preoperative level within one month, and censequently, thalamotomy seems practicable, since there were scarcely observed any severe side-effects in blood components. That most of changes due to thalamotomy stood in contrast to those following prefrontal lobotomy, was mentioned, in addition to some comments on mechanism of the changes.

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Studies on the Antibiotic Substances from Actinomyces. 15th Report.

(Preliminary note)

By

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1. Eight strains of Actinomyces belonging to streptothricin type (1 and 2) and the purified active HCl salts isolated from them were examined comparatively upon their " antibacterial spectra " using several kinds of test organisms including streptomycin resistant *E. coli* and 4 strains of *E. coli* which were made resistant to each of 4 different kinds of streptothricin respectively. All streptothricin resistant *E. coli* strains were resistant to each of HCl salt solutions of both types of streptothricins in a similar way, so it was not possible in this way to differentiate both types of streptothricins from each other. They were useful, however, in order to differentiate the culture of Streptomyces themselves, because the streptothricin resistant *E. coli* strains were somewhat differently subceptible according to their own types, even if they were far more resistant than the original strains of *E. coli*.

2) A strain of Streptomyces (O-1) showed a streptothricin-like spectrum in a state of culture on the plate, while the other strain (488) in a state of culture filtrate and finally the third strain (149) in a state of pure HCl salt solution, showed a similar spectrum. This fact indicated that there were many incomformities between the spectra produced by organisms, by their culture filtrates or by the solutions of isolated antibiotics, and may be explained by the assumption, that the antibiotics produced by Streptomyces culture on the plates may exceed both, qualitiatively and quantitatively that contained in their culture filtrates and may contain other antibiotics different from that yielded in a state of HCl salt solutions.

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On the Adrenaline Secretion of the Suprarenal Glands during the Insulin Hypoglycemia in Dogs, Anesthetized with Evipan-Sodium.*

By

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In the previous communication in conjunction with Dr. Kobayasi,[1] we have shown that the adrenaline secretion rate from the suprarenals in dogs does not materially alter in evipan-sodium narcosis.

It is further of interest for us to know, whether or not the evipan-sodium narcosis acts to inhibit the accelerating action upon the adrenaline secretion of some agencies. For the first time we have studied the influence of evipan-sodium anesthesia upon the acceleration by insulin of the adrenaline secretion.

The hyperadrenalinemia during the insulin hypoglycemia in dogs without narcosis has been studied quantitatively in our laboratory.[2] Insulin acts to accelerate the adrenaline secretion through the central mechanism.[3]

However in anesthetized animals, some authors obtained the negative outcome on the action of insulin upon the adrenaline secretion. Stewart and Rogoff[4] collected the suprarenal vein blood of cats under narcosis (ether or urethane and ether) by means of the cava-pocket method. In three cats they could not find any definite acceleration of adrenaline secretion after insulin application. With the same method, Rogoff and Nixon[5] affirmed this result in cats and dogs. After paralysing the thalamic centers by the injection of sodium barbital or a large dose of magnesium sulphate, Kettenmeyer and La Barre[6] found non-augmentation of adrenaline content in the suprarenal vein blood in dogs during the hypoglycemia due to the application of insulin in a dose of 0.5–1.0 unit per kg of body weight. They supported by this observation that the insulin hypoglycemia acts on the glycoregulator centers in the thalamic region.

* Shortly reported at XXII Annual Meeting of the Japanese Physiological Society, held at Fukuoka, March, 1943. Nihon Seiri. Z., 1943, **8**, 551. Jap. J. Med. Sci., Biophysics, 1944, **9**, 77.

It may be added here that in dogs, under avertin narcosis, H. Sato, Satow and Degti[7] in our laboratory observed the definite accelerated adrenaline secretion during the insulin hypoglycemia.

Method.

Three dogs were anesthetized by evipan-sodium. At first, the right saphena vein was uncovered under novocain-anesthesia and a small cannula for injection was inserted therein.

Evipan-sodium (Bayer) was then injected intravenously in a dose of 40-50 mg per kg of body weight as a 10% solution. The suprarenal vein was prepared through the lumbar approach for collecting the suprarenal vein blood. During the operation, evipan-sodium was injected additionally in a dose of 15–20 mg per kg. Left femoral artery for recording of the blood pressure tracings was also exposed.

About 2 hours after finishing the operation, the experiment was started. Some minutes before the start of experiment, evipan-sodium was again injected intravenously in a dose of 30–40 mg per kg. Evipan-sodium given in such a manner was sufficient to obtain a deep narcosis during the whole course of experiment.

Some blood specimens were collected and then insulin (Toronto) was injected intravenously in a dose of 7 units per kg of body weight. Blood samples were collected thereafter from time to time.

The estimation of adrenaline in the collected suprarenal vein blood was carried out by means of the rabbit intestine segment method. The blood sugar content was estimated by Hagedorn and Jensen. The arterial blood presurre was recorded, using a mercury manometer connected with the left femoral artery. The rate of heart beat and the frequency of respiration were counted from the blood pressure tracings. Body temperature and room temperature were measured from time to time.

Result.

Exp. 1. A femal dog anesthetized with evipan-sodium, 14.5 kg. The initial adrenaline secretion rate in this dog was 0.01 γ per kg per minute. 30 minutes after insulin injection the rate of adrenaline secretion increased to 0.09 γ. 15 minutes later it increased further and 0.24 γ per kg per min. was reached. Then it was going down. The blood sugar content, which was 0.080–0.082% before insulin, decreased markedly after insulin. It was estimated as 0.036% 20 minutes after insulin application and thereafter some undulations were observed. Notwithstanding the remarkable hypoglycemia, no hypoglycemic symptoms were found except the very slight tremor, which was seen one hour after insulin.

Exp. 2. A male dog anesthetized with evipan-sodium, 11.6 kg. Before insulin, the adrenaline secretion rate was 0.01 γ per kg per minute.

50 minutes after insulin it was found to have increased to 0.13 γ. The blood sugar decreased from 0.089–0.093% to 0.049% 50 minutes after insulin. Also in this dog, no visible symptoms were observed during hypoglycemia.

TABLE I.

Effect of Insulin on the Adrenaline Secretion in Dog Anesthetized with Evipan-sodium.

Time before or after injection of insulin (min.)	No. of specimen	Quantity of blood (cc)	Duration of collection (sec.)	Blood flow per kg per min. (cc)	Adrenaline in 1cc blood (γ)	Adrenaline secretion per kg per min. (γ)	Blood sugar (%)	Blood pressure (mm Hg)	Heart rate (per min.)	Respiration (per min.)	Body temperature (°C)	Room temperature (°C)
Exp. 3 12. II. 1943 Dog 12.2 kg ♂												
10:40 a.m. Evipan-sodium (40 mg per kg) injected intravenously.												
10:45 a.m.—0:45 p.m. Left lumbar route and left femoral artery preparation.												
11:50 a.m. Evipan-sodium (15 mg per kg) injected intravenously.												
3:00 p.m. Evipan-sodium (30 mg per kg) injected intravenously.												
before 25	1	2.1	60	0.17	0.1	0.02	0.073	90	150	14		
	2	2.5	60	0.20								
10	3	2.9	60	0.24	0.075	0.02	0.080	85	150	14	37.5	12
	4	2.9	60	0.24								
3: 35 p.m. Insulin (7 units per kg) injected intravenously.												
after 5							0.080					
10							0.067					
15	5	1.7	30	0.28	0.3	0.08	0.049	82	138	24	37.0	12
	6	1.9	30	0.31								
20							0.044					
30	7	1.7	30	0.28	0.4	0.11	0.046	75	120	18	36.6	12
	8	1.8	30	0.29								
45	9	1.6	30	0.26	0.4	0.10	0.054	72	120	21	36.4	12
	10	1.7	30	0.28								
60	11	1.5	30	0.25	0.6	0.15	0.042	70	111	15	36.1	11
	12	1.5	30	0.25								
75	13	1.4	30	0.23	0.6	0.14	0.046	72	87	7	35.9	11
	14	1.5	30	0.25								
90	15	1.6	30	0.26	0.6	0.16	0.033	72	87	8	35.7	11
	16	1.7	30	0.28								

Exp. 3. A male dog anesthetized with evipan-sodium, 12.2 kg. The protocol is reproduced as an example. The basal rate of adrenaline secretion in this dog was 0.02 γ per kg per minute. After insulin, the rate of secretion was found to increase and measured as 0.08 γ per kg per minute 15 minutes after injection, 0.11 γ 30 minutes, 0.15 γ 60 minutes, 0.14 γ 75 minutes and 0.16 γ 90 minutes after insulin. The blood sugar decreased from 0.073–0.080% to 0.049% 15 minutes after insulin and there-

after some variations were observed. In this dog no hypoglycemic symptoms were observed, too.

From the results above quoted it is clearly demonstrated that in dogs anesthetized with evipan-sodium insulin hypoglycemia acts always to accelerate the adrenaline secretion of suprarenals. The magnitude of accelerated secretion of adrenaline corresponds roughly to that noted in Dog. 6 of Yen, Aomura and T. Inaba, which received the same dose of insulin with us but without narcosis. In their dog the maximum acceleration of adrenaline secretion after 7 units per kg insulin was 0.20 γ per kg per minute.

Concerning the blood sugar, insulin hypoglycemia in dog under evipan-sodium narcosis is somewhat severe in comparison with that in non-anesthetized dog. Nevertheless no visible hypoglycemic symptoms were found in our dogs under evipan-sodium in the course of so severe hypoglycemia.

Summary.

In three dogs, which were anesthetized with evipan-sodium, the suprarenal vein blood was collected through the lumbar approach. Adrenaline in the blood samples was estimated by means of the rabbit intestine segment method. The blood sugar and blood pressure were also measured simultaneously.

Insulin was injected in a dose of 7 units per kg of body weight. During the insulin hypoglycemia, the adrenaline secretion rate was accelerated here in an almost the same degree as in the dog without narcosis.

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Biochemical Studies on Carbohydrates.

CXLV. N-Acetylchondrosamine, N-Acetyl-α-methylchondrosaminide and Desaminochondrosin-cinchonidine-salt.

By

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i) N-Acetylchondrosamine was first synthesized by Masamune and Osaki (1943)[1] and then, in a similar manner, by Stacey (1944)[2]. The preparation of the former authors melted at 154° C, while that of the latter at 120–122° C. A product which we have obtained melts at 159–160° C. Masamune and Osaki gave no information as to the amount of crystal water. Although Stacey noted that his substance contained 1 mole crystal water, exposure of ours to 105° C and low pressure reduced only 1.55% weight, which appeared to be no crystal water but mere moisture. With respect to the optical rotation also, our substance ($[\alpha]_D^{\infty}=+94.5°$ at equilibrium) diverges from Stacey's ($[\alpha]_D^{\infty}=+80°$ at equilibrium)[3].

ii) N-Acetyl-α-methylchondrosaminide, which Stacey[2] prepared by boiling α-pentacetylchondrosamine in methanol containing 2% HCl, melted at 217–218°C and possessed $[\alpha]_D^{\infty}$ of 170° C in chloroform.[3] W subjected β-pentacetylchondrosamine to a similar procedure. Recrystallization from methanol of the crude product by the aid of ether did not raise the melting point above 203–204°C, but after following purification by means of alcohol, the substance melted at 219° C as Stacey's. Nevertheless, it contains ½ mole H_2O as crystal water and does not dissolve in chloroform. Its $[\alpha]_D^{\infty}$ is +187.3° in water.

iii) Cinchonidine salt of desaminochondrosin. Chondrosin hydrochloride (Levene),[4] a HCl-hydrolysis product of chondroitinsulfuric acid, was deaminized according to Levene and the amorphous product was changed into crystals with cinchonidine. Nagaoka[5] recommended the brucine salt as readily crystallizable, but cinchonidine appeared to us more suitable for isolation of the compound. The cinchonidine salt contains 1½ moles H_2O as crystal water. F.P., 194° C. Equilibrium $[\alpha]_D^{\infty}$ is $-66.5° \left(=\frac{-1.09\times 100}{1.64\times 1}\right)$ in water. Molisch, negative. Schiff,

positive.

On the other hand, chondrosinethylester hydrochloride (Hebting)[6] which is prepared from the disaccharide obtained by oxalic acid-hydorlysis of chondroitinsulfuric acid, was deaminized and after splitting the ester linkage, changed into cinchonidine salt. The product here has F.P. of 194° C similar to the above, and the mixture of the both preparations showed no depression of melting point, which offers a proof that oxalic and hydrochloric acid cleave the same glucosidic linkages of chondroitinsulfuric acid. Trials of preparing brucine-, cinchonine- and strychinine-salt were unsuccessful.

Experimental.

N-Acetylchondrosamine. Chondrosamine hydrochloride (Levene) was acetylated with acetic anhydride and silver acetate as was by Masamune and Osaki.[1] The filtrate from AgCl was diluted with an equal volume of methanol, and ether and petrol ether were added carefully to obtain crystalline deposits. From 2 g. of the starting material was yielded 0.87 g. of a crude product (43.3% of theory). Reprecipitation from methanol by the aid of ether was effected three times, whereby sometimes crystals in thin needles and sometimes congregates of petaloid crystals were given. The final product took the latter form. The amount of substance diminished to 360 mg. It showed the positive indirect-Osaki-Turumi reaction and reduced ammoniacal silver nitrate solution (Tollens) at 70° C. 1.55% weight was lost at 105° C under reduced pressure, and analysis and measurement of the optical rotation was conducted on the moisture-free substance.

Total N (micro Kjeldahl).

3.072 mg. substance, 1.362 cc. 0.01 *N* acid neutralized.

$C_8H_{13}O_6N$. Calc., 6.33%; found, 6.21%.

NH_2-N (micro Van Slyke).

9.21 mg. substance, no N_2 evolved.

CH_3CO (Suzuki).

6.109 mg. substance, 2.93 cc. 0.01 *N* $Na_2S_2O_3$ used.

$C_6H_{12}O_5N(COCH_3)$. Calc., 19.5%; found, 20.6%.

Iodine value (Macleod-Robison).

2.86 mg. substance, 1.3 cc. 0.02 *N* iodine used.

$C_7H_{14}O_5N\cdot CHO$. Calc., 90.5; found, 90.9.

$[\alpha]_D^{\infty}$ at equilibrium $= \frac{+1.74\times 100}{18.41\times 1} = +94.5°$ (in water).

At the 5th minute after dissolution of the substance it was +145.0° ($\alpha = +2.67°$).

N-Acetyl-α-methylchondrosaminide. Crude β-pentacetylchondrosamine was prepared according to Hudson and Dale,[7] that is, the precipitates appearing on neutralization of the diluted reaction-mixture were washed

thoroughly with water and dried. And the amorphous product was immediately treated by the method of Moggridge and Neuberger[8] for synthesizing N-acetylmethylglucosaminide from pentacetylglucosamine, as follows. To 1.65 g. of the material 36 cc. of 2.2% methanol-HCl were added and refluxed for 2 hours. The substance dissolved in 1 hour completely. When cold, the solution was neutralized with 3.5 g. of lead carbonate and filtered and ether added slowly. The crystalline deposits were brought onto a suction funnel, washed with a small volume of a methanol-ether (1:1 by volume) mixture and dried in air. The crop at this stage was 350 mg. Purification was processed by crystallization from methanol by the aid of ether as above three times, and 161 mg. of a product were given as non-colored, thin needles with a melting point of 203–204° C. Since, in the third time, the melting point was not raised anymore, 150 mg. of it were taken up in 30 cc. of hot abs. ethanol and allowed to crystallize out by placing in an ice-chest for one day. The deposits (needles) here were washed with abs. alcohol and ether. They amounted to 60 mg. F.P., 218–219° C. A further repetition of this procedure did not elevate the melting point (219°C). The final yield, 31 mg.

The product did not dissolve in chloroform. The indirect-Osaki-Turumi and Tollens test were negative. Analysis:

Crystal water.
5.534 mg. substance lost 0.197 mg. H_2O at 105°C under diminished pressure.
$C_9H_{17}O_6N+\frac{1}{2}H_2O$. Calc., 3.69%; found, 3.56%.
N (micro Kjeldahl).
4.08 mg. substance, 1.67 cc. 0.01 *N* acid neutralized.
$C_9H_{17}O_6N+\frac{1}{2}H_2O$. Calc., 5.74%; found, 5.73%.
NH_2-N (micro Van Slyke).
10.75 mg. substance, no N_2 evolved.
CH_3CO (Suzuki).
6.231 mg. substance, 2.62 cc. 0.01 *N* $Na_2S_2O_3$ used.
$C_7H_{14}O_5N\cdot COCH_3+\frac{1}{2}H_2O$. Calc., 17.62%; found, 18.08%.
Opitical rotation in water:

$$[\alpha]_D=\frac{+3.82\times 100}{2.04\times 1}=+187.3^\circ \text{ (no mutarotation).}$$

Desaminochondrosin cinchonidine salt.

1) From chondrosin hydrochloride (Levene). a) Desaminochondrosin. Staring with chondrosin hydrochloride (Levene), desaminochondrosin was prepared by further following Levene with minor modification: — 6 g. of the material (a white powder) were taken up in 150 cc. of water, shaken in a brown flask with 2.4 g. $AgNO_2$ for 2 hours at room temperature (6°C) and stood in a thermostat of 37° C, with occasional shaking, for 6 hours. AgCl was filtered off and washed with 30 cc. of hot water. The filtrate and washing were refluxed together, protecting from sunlight, for

2 hours. Then the browned solution was allowed to stand overnight (0–3° C), transferred to a brown flask, and after addition of 10 cc. of 0.1 *N* HCl and 1.2 g. $AgNO_2$, stood for 1 hour at room temperature, for 6 hours in the thermostat, for 2 hours on a water bath, and overnight at room temperature in sequence. The mixture was again filtered and the lump on the funnel was washed with 30 cc. of hot water. And the filtrate and washing were bubbled with H_2S and the deposits were separated and washed with hot water as above. The filtrate and washing here were distilled in vacuo to a syrup, 50 cc. acetone added and the precipitates washed with acetone and dried in a vacuum desiccator. 3.7 g. of a hygroscopic yellowish powder (amorphous) were given.

b) Cinchonidine salt. 3.5 g. of the product, 5 g. of cinchonidine and 150 cc. of 80% methanol were warmed together (under reflux) on a water-bath for 30 minutes. The dark brown solution was cooled (Some crystalline deposits were rejected; pH of the solution was 8.2) and distilled in vacuo to about 50 cc. The precipitates were separated and washed with 20 cc. of water. Then the washing and mother liquid were united and condensed furtheron to dryness, and the residual was exhausted with changes of water (in total 50 cc.). The extracts (yellowish brown; pH: 4.6) were treated with chloroform many times until a chloroform portion renewed got no more colored, concentrated by vacuum distillation to somewhat syrupy consistency and placed in an ice chest whereupon abundant crystals came out in thin needles. After 2 days the crystals were collected on a funnel with suction after quick agitation with 5 cc. of ice-cold water, and further washed with 20 cc. of water, with 10 cc. of abs. alcohol and with 30 cc. of chloroform. The supple needles with yellow tint were dried in air. Their crop was 480 mg. F.P. 193–194° C.

1.4 g. of the crude salt obtained as above were dissolved in 120 cc. of water by slight warming (In cold water it was difficultly soluble), removed from a small quantity of insoluble contaminants and distilled in vacuo to about 2 cc. with following placing in an ice-chest. After 1 day, 5 cc. of ice-cold water were added to the crystalline porridge and the crystals filtered off were washed with water, abs. alcohol and chloroform. The yield of a faintly yellow-colored product (thin needles) was 1.1 g. It melted sharp at 194° C (Another recrystallization could not raise the melting point). Molisch and Elson-Morgan, negative. Goldschmiedt and Neuberg-Saneyoshi, positive. Hot Fehling solution was reduced. Analysis:

Crystal water.

32.6 mg. substance lost 1.4 mg. H_2O at 100–110°C under diminished pressure. $C_{12}H_{18}O_{11}\cdot C_{19}H_{22}ON_2+1\frac{1}{2}H_2O$. Calc., 4.1%; found, 4.3%.

N (Pregl's micro Kjeldahl method).

5.0 mg. substance, 1.503 cc. 0.01 *N* acid neutralized.

$C_{31}H_{40}O_{13}N_2+1\frac{1}{2}H_2O$. Calc., 4.25%; found, 4.21%.

NH_2-N (micro Van Slyke).

16.6 mg. substance, no N_2 evolved.

Iodine value (Macleod-Robison).

8.04 mg. substance, 1.3 cc. 0.02 *N* iodine used.

$C_{11}H_{17}O_{10}(CHO)\cdot C_{19}H_{22}ON_2+1\frac{1}{2}H_2O$. Calc., 30.4; found, 32.3.

Optical rotation in water.

$$[\alpha]_D^{\text{t}} = \frac{-1.09\times 100}{1.64\times 1} = -66.5^\circ \text{ (no mutarotation)}.$$

2) From chondorsinethylester hydrochloride (Hebting). 1 g. of the meterial, which had been prepared according to Hebting from chondroitinsulfuric acid, was deaminized similar to above, and 0.7 g. of amorphous desaminochondrosinethylester was obtained whose crystallization was attempted in vain. Then 0.6 g. of the substance was taken up in 15 cc. of water and the saturated $Ba(OH)_2$ added to a pH of 9.0. On standing in ice for 3 hours the solution became less alkaline (to pH 8.0), so that additional baryta was added to the original pH. After 1 day barium was carried down quantitatively with sulfuric acid and the filtrate was distilled in vacuo to about 1 cc. and added to with acetone. 0.4 g. was desaminochondrosin (an amorphous hygroscopic powder) produced. This was changed into cinchonidine salt in the above manner. The product (noncolored) here amounted to 80 mg. It possessed F.P. of 194° C and no depression of melting point occurred when its mixture with the product in 1) was heated.

Through the Grant Committee of the Science Council was given a grant from the Education Department which enabled us to carry out this work. H. M.

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Studies on the Antibiotic Substances from Actinomyces. 16th Report.

(Preliminary note)

By

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It is already well known, that an antagonistic streptomyces has a characteristic property to grow abundantly on a nutrient agar, which contains an antibiotic produced by the same streptomyces in such an amount that the other strains of streptomyces can not grow well.

This fact lead us to an idea of an additional use of streptomyces to "antibacterial spectrum," namely to the cross-inhibition test between streptomyces themselves.

Fifteen cc of 1 per cent glucose nutrient agar was distributed in Petri dishes each, and after solidification, a strain of inhibitory streptomyces was streaked diametrically in a straight line, and incubated for 3 days at 27°C. Then many strains of the test streptomyces including own strain were streaked at the side of the growing streptomyces perpendicular. After 3 days incubation, the lengths of the inhibition zones was measured in mm.

Ten representative strains of active streptomyces, viz., streptomycin-, streptomycin-like substance-, streptothricin type 1-, 2- and 3-, chloramphenicol-, aureomycin-, special substance Nos. 1-, 2- and 3-producing strains were examined in the way mentioned above. From the results, it was concluded as follows:

1. All strains which produce specific antibiotics respectively, do not inhibit their own strains as a rule, while they inhibit the other strains more or less according to their similarities.

2. However, there are some exceptions from these fundamental principles.

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Chemistry and Biology of Lipids.

VI. Group A Lipoid from Stomach Mucosa of Pig.

By

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Mucosa batches from 20 pig stomachs were treated with hot abs. alcohol separately after dehydration (with cold alcohol). All the extracts obtained were found to contain Group A lipoid or lipoids. Hence, next, for their isolation, mucosa batches from 10–20 stomachs were employed together at one time. By successive fractionation of the hot-abs. alcoholic extracts by the aid of organic solvents, taking the anti-iso-hemoagglutinative force as an indication, were finally obtained two kinds of preparation, which diverged from each other in the sero-reactivity as well as N-, P- and carbohydrate-content. Of them the less active (Prep.-I_{sol}'s) appeared to be a mixture of the more active (Prep.-II_{sol}'s) that was probably nearly pure, a faintly active, almost carbohydrate-free lipoid (Prep. I'_{sol}-Sol) and other lipids. The approximate equivalent ratio of N to P was 2.5–2.6 : 1 in II_{sol}'s, 2.7–2.8 : 1 in I_{sol}'s, and 3.0 : 1 in I'_{sol}-Sol. II_{sol}'s were richer in carbohydrate than in I_{sol}'s. I_{sol}'s inhibited iso-agglutination of A erythrocytes at a dilution of $1:2.56–5.12\times10^4$, II_{sol}'s at a dilution of $1:1.024–2.048\times10^5$, and I'_{sol}-Sol at a dilution of $1:3.2\times10^5$.

Experimental.

Preparation of the Group Lipoids.

Fractionation was carried out taking the group activity as an indication (2.5–10 mg. of substance were employed at one time for the assay). An example will be given.

1) Mucosa of 13 pig stomachs was torn from muscularis externa and after scraping off the mucus, minced together. The obtained batch weighing 2100 g. was stood with 5 volumes of 91% alcohol for 10 days, then separated and dried in a Faust apparatus. The residue amounted to 449 g. This was boiled under reflux with 5 and 3.5 volumes of 95% alcohol for 1 and 1/2 hour respectively in succession. Each time the ex-

tract was separated by decantation and squeeze while hot. From the yellowish transparent fluids combined (in total 3850 cc.), when cold, came out non-colored, amorphous and crystalline deposits, which were centrifuged after standing for days and set aside (a hygroscopic powder tinged yellow and amounting to 6.2 g.)—**Div. II.** The supernatant fluid was distilled in vacuo. The brown thick syrup that remained (2.5 g.)—**Div. I**—were kept under 100 cc. of acetone overnight and the insoluble part was centrifuged and placed in a vacuum desiccator (Acetone turned yellowish brown and left 4.5 g. of a brown oil on evaporation). In the next, fractionation with alcohol was effected. Namely, the acetone-insoluble substance (5 g.) was treated with 30 cc. portions of abs. aclcohol until no colored matter came out, and dried—a hygroscopic yellowish powder, 2 g. And the washings were distilled together to a thick solution (about 2 cc.), 30 cc. of acetone added and the precipitates centrifuged and dried—a brown gelatinous tuff clump, 3 g. On agitating with abs. alcohol (30 cc.) it remained partially undissolved. The centrifuged supernatant liquid was again condensed and precipitated, and the centrifugate dried (2 g.) was once more treated with alcohol to recover the final trace of the insoluble part. All the alcohol-insoluble were united (a coarse yellowish powder, 2.7 g.) and removed from the ether-soluble contaminants (1 g.) by washing with three 30 cc. portions of ether. After further washing with 30 cc. of acetone and drying, it was finally subdivided into parts soluble and insoluble in hot benzene. Here the substance was boiled with 30 cc. of benzene at one time and centrifuged while hot. This operation was repeated thrice. The insoluble white powder obtained amounted to 0.7 g. and the soluble brownish brittle clump (**Prep. I_{sol}**) to 0.8 g. The properties of the fractions above will be noted below together with those of the corresponding fractions obtained by another similar run.

2) Div. II in 1)—the deposits that had occurred from the hot-alcoholic extracts of the tissue when cold—was fractioned with acetone, ether, hot benzene, water (faintly alkalized) and hot alcohol in turn:—a) The substance was washed with 30 cc. portions of acetone with centrifugation, until no colored material came out. b) The hygroscopic amorphous powder after drying up in a vacuum desiccator (3 g.) was stirred up with 25 cc. of dry ether. The centrifuged supernatant fluid remained turbid. Hence, 2 cc. of abs. alcohol were added, and the mixture agitated was again centrifuged, whereby a transparent yellowish supernatant fluid was given. The centrifugate was dried as above—an amorphous grayish powder weighing 2 g. c) The ether-insoluble part immediately above was then refluxed with two 20 cc. portions of benzene. The second portion of benzene was not colored. The extracts, decanted while hot, were distilled under diminished pressure to dryness. 0.8 g. of a brownish,

hard, gelatinous substance was left. d) Its water-soluble fraction was separated by agitation with 20 cc. of water and a few drops of 0.1 *N* NaOH added to a pH of about 8.0 and centrifugation four times. The supernatant fluids (80 cc. in total), which were opalescent, were vacuum-distilled, after acidulation (to pH 5.8) with 0.1 *N* HCl, to a thick solution, and precipitated with acetone. The flocculent deposits were centrifuged and dried. Yield 0.68 g. e) Of the substance 0.2 g. was boiled with abs. alcohol over and over again, each time using 30 cc. of the solvent and refluxing for 15 minutes. The hot extracts were decanted out and distilled together to dryness (150 mg.)—**Prep. II_{sol}**. The residue unextracted was 30 mg. —**Prep. II_{ins}**.

3) Prep.-II_{sol}'s contained more than 20% carbohydrate and were more sero-reactive (active at a dilution of $>1.024\times10^5$) than Prep.-**I_{sol}'s**, whose carbohydrate content reached only a half. Therefore, it was tried to obtain from Prep. **I'_{sol}**, a preparation by a run similar as described in 1) (carbohydrate as glucose, 11.9%; the dilution where anti-iso-hemoagglutinative force manifested itself, $1:5.12\times10^4$), a substance resembling Prep.-II_{sol}'s by application of the last-stage process of 2) with hot alcohol, as follows. 0.2 g. of Prep. I'_{sol} was boiled with four 20 cc. portions of abs. alcohol. The insoluble brown residue (10 mg.) thereby will be referred to as **Prep. I'_{sol}-Ins.** From the hot extracts combined, deposits appeared when cold, which were centrifuged and dried—**Prep. I'_{sol}-$Prec_1$** (a gray powder, 30 mg.). The supernatant fluid was distilled in vacuo and the remainder (a white powder, 150 mg.) was further subjected to a similar treatment twice (First time: abs. alcohol used, one 30 cc. portion; the hot-alcohol-insoluble part, 20 mg.; the precipitates that appeared on cooling, 30 mg. Second time: abs. alcohol used, one 15 cc. portion; the insoluble part, a trace; the precipitates in the cold, 25 mg.). The final evaporated residue—**Prep. I'_{sol}-Sol** (a white powder, 60 mg.) —was found to contain only 1.5% carbohydrate as glucose and its anti-agglutinative activity was very week. Prep. I'_{sol}-$Prec_1$, which was active even at a dilution of $1:4\times10^5$, possessed no more than about 13% carbohydrate, diverging remarkably from Prep.-II_{sol}'s. Thus the serological potency of preparations had apparently no close connection with the carbohydrate content, but it is possibly due to the co-presence of some activating or inhibiting lipids (Cf. Hamasato[1] regarding the presence of activating substances).

Properties of the Fractions.

1) Serological potency.

It was determined by our usual procedure, using a minute quantity

Table I.

Anti-iso-hemoagglutinative Potency of Franctions of Lipids Extracted from Pig Stomach Mucosa with Hot Alcohol.

The fractions are named as in the text. B sera had agglutinin titre 320 and were diluted to 1:20 for the assay, A sera had either titre 160 or titre 80 and were diluted to 1:10 or to 1:5.

—: no agglutination, ±: faint aggl., +: slight aggl., ++: marked aggl., +++: strong aggl.

Fraction	Group of erythro-cytes	Dilution of substances														Agglutinin titre of A sera
		1 × 10²	2 × 10²	4 × 10²	8 × 10²	1.6 × 10³	3.2 × 10³	6.4 × 10³	1.28 × 10⁴	× 2.56 × 10⁴	× 5.12 × 10⁴	× 1.024 × 10⁵	× 2.048 × 10⁵	× 4.096 × 10⁵	× 8.192 × 10⁵	
Prep. I_{sol}	A	—	—	—	—	—	—	—	—	—	+					160
	B	+	+	++	++	++	++	++	++	++	++					
Prep. I'_{sol}	A	—	—	—	—	—	—	—	—	—	—	±	+			160
	B	+	+	++	++	++	++	++	++	+++	+++					
Prep. II_{ins}	A		—	—	—	—	—	±	+	+	+					80
	B		+	+	+	++	++	++	++	++	++					
Prep. II_{sol}	A		—	—	—	—	—	—	—	—	—	—	±			80
	B		—	+	+	++	++	++	++	++	+++	+++	+++			
Prep. II'_{sol}*	A						—	—	—	—	—	—	—	±		160
	B						+++	+++	+++	+++	+++	+++	+++	+++		
Prep. I'_{sol}-Ins	A		—	—	—	—	—	—	+	++						
Prep. I'_{sol}-$Prec_I$	A			—	—	—	—	—	—	—	—	—	—	—	±	
Prep. I'_{sol}-Sol	A			—	—	—	—	+	+	+	+	++	++	++	++	

* In the run, whereby this fraction was obtained, fractionation with alkaline water was not effected.

TABLE II.

Solubilities of Fractions of Lipids Extracted from Pig Stomach Mucosa with Hot Alcohol.

The fractions are named similarly as in Table I.

−: insoluble, ±: faintly sol., +: partly sol., ++: mostly sol., +++: completely sol.

Fraction	Solvent															
	Acetone		Abs. alcohol		Benzene		Chloroform		Ether		Glacial acetic acid		Petrol ether		Tetralin		Water	
	cold	hol	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot
Prep. I_{sol}	−	−	−	±	+++	+++	+++	+++	−	−	−	−	−	−	−	+++	++†	++
Prep. I'_{sol}	−	−	−	−	+++	+++	+++	+++	−	−			−	+	−	+++	+‡	+
Prep. II_{ins}			−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+
Prep. II_{sol}			−	+	+++	+++	+++	+++	−	−	−	−	−	−	++	+++	++	++
Prep. II'_{sol}*	−	−	−	−	−	+++	−	+++	−	−			−	−	−	++	+++	+++
Prep. I'_{sol}-Ins			−	−	−	+	−	+	−	−	−	−	−	−	−	+	++	+++
Prep. I'_{sol}-Sol			−	+++	++	+++	++	+++	−	−	−	−	−	−	−	+++	+	++

* Signifies the same as in Table I.

† Dissolves transparently on alkalization to pH 9.0.

‡ ++ when faintly alkalized.

TABLE III.

Composition of Fractions of Lipids Extracted from Pig Stomach Mucosa with Hot Alcohol.

Names of the fractions accord with corresponding names in Tables I and II.

Fraction	In per cent						In equivalents per equivalent weight	
	N	P	Total carbohydrate as glucose	Hexose† as galactose	Hexosamine† as glucosamine	Ash	N	P
Prep. I_{sol}	2.8	2.3	13.4	6.4	7.3	6.6	2.7	1.0
Prep. I'_{sol}	2.9	2.2	11.9	6.4	6.0	6.8	2.8	1.0
Prep. II_{ins}			6.8			6.9		
Prep. II_{sol}	2.1	1.8	21.8			11.1	2.6	1.0
Prep. II'_{sol}*	2.3	2.0	23.1			7.9	2.5	1.0
Prep. I'_{sol}-Ins			12.9			15.3		
Prep. I'_{sol}-$Prec_1$	3.2	2.5	13.3			4.2	2.8	1.0
Prep. I'_{sol}-Sol	4.9	3.6	1.5			5.9	3.0	1.0

* Signifies the same as in Tables I and II.

† The hydrolystates gave the positive Elson-Morgan reaction in addition to the positive Molisch.

of NaOH, when necessary, to form a homogeneous solution of the substances in saline (Sometimes was given only a turbid solution). The results are embodied in Table I. The substances named same but marked with and without apostrophe signify corresponding fractions in different similar runs.

2) Solubility.

Table II illustrates the solubilities of several fractions.

3) Composition.

Analytical methods.

N: Micro Kjeldahl.

P: Haneda, Sinokawa and Yasuoka's modification of Plimmer method.

Carbohydrate: Hydrolysis of the substances was effected by heating with 1 N H_2SO_4 for 4 hours in an ampoule and the hydrolysates were filtered, when cold, and after neutralization with 2 N KOH, analyzed on the reducing force according to Masamune and Tanabe[2]. (The filtrates contained no split products precipitable by phosphotungstic acid.) The values obtained are expressed as "total carbohydrate" in Table III. In some of the preparations, fractionated analysis of hexosamine and hexose, too, was carried out by the aid of mercuric acetate ("the first method" of Masamune and Tanabe[2]).

Table III shows te figures obtained.

Of Prep. II'_{sol}, a portion (150 mg.) was boiled with alcohol over again and after removal of the insoluble part (30 mg.), the hot solution was distilled. However, the substance recovered was found almost unaltered not only in the total carbohydrate content (22.0%) [ash, 7.4%] but also in the anti-iso-hemoagglutinative potency (active at a dilution of $1:4.096\times10^5$), suggesting that Preps. II_{sol} and II'_{sol} represent the approximately pure form.

Examination of 20 Pig Stomachs as to the Group Activity of the Mucosa Lipids.

Mucosa batches obtained from 20 pig stomachs were separately subjected to incipient treatments in the preparation procedure (described above). And the fractions corresponding to Div. I were washed with acetone and after drying, examined regarding the sero-reactivity which gave the results in Table IV. Pigs each possess Group A lipoid or lipoids in the gastric mucosa but not Group B lipoid.

Summary.

Group A-active preparations of lipid nature have been otbained from the mucosa of pig stomachs and their potency, solubility and composition were investigated. Also trials to disclose the nature of the pure group lipoid from the present source were made.

Table IV.

Group Activity (anti-iso-hemoagglutinative) of Mucosa Lipids of 20 Pig Stomachs.

To A serum the fractions tested were all inert at a dilution of 1:100, except that of Pig No. 10, which was active at that dilution though not at the doubled dilution. B and A serum used had agglutinin titre 320 and 80 and were diluted to 1:20 and 1:5 respectively.

Pig No.	Weight of mucosa (g.)		Acetone-insoluble part of Div. I*	
	Fresh	Dehydrated	Weight (g.)	Group A-active dilution (times)
1	160	32	0.2	800
2	120	25	0.1	800
3	150	29	0.1	800
4	255	50	0.7	1600
5	118	23	0.1	200
6	113	22	0.1	1600
7	190	35	0.2	1600
8	228	44	0.2	1600
9	238	41	0.4	1600
10	208	37	0.5	100
11	208	38	0.25	1600
12	283	50	0.4	200
13	226	37	0.4	1600
14	210	36	0.2	1600
15	193	30	0.1	400
16	233	41	0.2	400
17	162	29	0.2	1600
18	266	50	0.3	800
19	227	45	0.3	800
20	267	52	0.5	1600

* The lipids remaining in solution when the hot-alcoholic extracts of the mucosa were allowed to cool.

Through the Grant Committee of the Research Council was given a grant from the Education Department in aid to us. H. M.

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Chemistry and Biology of Lipids.

VII. Do the Blood Group Lipoids of Animal Organs Belong to Parenchyma or Connective Tissue?

(A Supplementary Note to Our Cancer Studies)

By

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In his work on water-extractible high-molecular tissue components, Masukawa,[1] one of the writers, set aside the hot-alcoholic extracts of the materials. The present account deals with the experiments undertaken to the same end on the blood group lipoid in those extracts. As was reported in that article, the author divided the mucous membrane of pig stomachs treated with alkali into two parts, mucosa propriae together with surface epithelium on one hand and submucosa on the other, and treated the batches of those parts separately with hot alcohol after dehydration with cold alcohol. Here the extracts were subjected to like fractionation by the aid of organic solvents as the lipids from the entire mucosa.[2]

From the submucosa extract were given only faintly active subdivisions and the most active could not inhibit the iso-agglutination of A erthrocytes when it was diluted more than 400 times and was small in amount besides. On the contrary, from the extract of the cell-rich tissue portions strongly active products were yielded in relatively large quantities. Thus it was concluded that the blood group lipoids, too, are constituents of parenchymal cells.

Experimental.

The materials.

The fresh mucous membrane = surface epithelium, mucosa propriae, muscularis mucosae and submucosa altogether (mucus is removed): 1543 g.

Mucosa and surface epithelium, soaked with alkali, torn off from submucosa and dehydrated: 135 g.

Submucosa, dehydrated: 128 g. (Cf. Masukawa[1])

I. Group A-active Fractions of the Lipids from Mucosa propriae and Surface Epithelium of Pig Stomachs.

Fractionation of the hot-alcoholic extract.

The material dehydrated (135 g.) was refluxed with 5 volumes of alcohol and the extract (625 cc.) was stood overnight (Regarding details, see Masukawa[1]). The deposits, that had appeared in the cold, were separated and after washing with 20 cc. of alcohol, dried—**Div. II** (0.6 g.).

1) a) The mother fluid and washing of the deposits were distilled together, then, to the syrupy residue—**Div. I**—80 cc. of acetone added and the precipitates washed with acetone well and dried. 1.3 g. of a yellowish coarse powder (hygroscopic) were given. b) This acetone-insoluble substance was stirred up in 30 cc. of abs. alcohol and centrifuged. The non-colored centrifugate weighed 1.1 g. when dried. The supernatant fluid was distilled and the brown gelatinous residue (0.23 g.) was washed with acetone (The present washing left nothing on evaporation) and again treated with abs. alcohol in a similar manner. The insoluble substance here amounted to 120 mg. (a hygroscopic non-colored powder). c) The two alcohol-insoluble portions were united and washed with acetone (30 cc.) and then after thorough drying, divided into the ether-soluble and -insoluble fractions, by exhausting with 20 cc. of dry ether three times. The former fraction, which was not more than 10 mg., inhibited the iso-agglutination of A erythrocytes only at a dilution of 1:200, whereas the latter (hygroscopic and non-colored) at a dilution of 1:1600—**Fr. $Et_{ins.}$** d) The ether-insoluble fraction, washed with acetone and dried, was boiled under return condenser with 20 cc. of benzene for 30 minutes and centrifuged while hot. The supernatant fluid was allowed to cool, whereby precipitation occurred, and after 1 hour, the deposits were separated by centrifugation. The amounts of the hot-benzene-insoluble (**Fr. Hbz_{ins}**, a white powder), the deposits on cooling (**Fr. Cbz_{ins}**, a yellowish powder) and the substance recovered from the mother liquid (**Fr. Cbz_{sol}**, a hygroscopic, yellowish, gelatine-like solid) were 800, 70 and 180 mg. respectively. The dilutions of them where they were group-active were 1:800, 1:6400 and 1:40000.

2) After washing Div. II with 20 cc. of acetone and drying, there remained 0.3 g. of a white powder. It was first fractioned with 30 cc. of benzene (slightly warmed). The part that had dissolved was recovered by evaporation of the solution to dryness—a light brown solid, 160 mg. —and then further agitated with dry ether. 120 mg. was the substance insoluble in ether—**Fr. Et_{ins}** (a white powder)—which exhibited the sero-reaction at a dilution of $1:8\times10^4$.

The mucosa used in the present experiment had been soaked with

dilute alkali into which the group lipoid had possibly passed over in part, so that the crops of the active fractions would have amounted more if mucosa without the alkaline treatment had been accessible.

Properties of the group-active fractions.

1) Specific anti-iso-hemoagglutinative potency. Table I shows the results in detail. The procedure employed was the usual to us.

TABLE I.

Anti-iso-hemoagglutinative Force of the Group-active Lipid Fractions of Mucosa propriae together with Surface Epithelium.

Fractions are named as in the text. A and B serum had agglutinin titre 80 and 160 and were diluted 5 and 10 times respectively.
−: no agglutination, ±: faint aggl., +: slight aggl., ++: marked aggl., +++: strong aggl.

Fraction	Group of erythroc.	Dilution of substances												
		×1 ×10²	×2 ×10²	×4 ×10²	×8 ×10²	×1.6 ×10³	×3.2 ×10³	×6.4 ×10³	×1.28 ×10⁴	×2 ×10⁴	×4 ×10⁴	×8 ×10⁴	×1.6 ×10⁵
Div. I-Fr. Et_{ins}	A	−	−	−	−	−	+	+	++				
	B	+	+	++	++	++	++	++	++	+++			
Div. I-Fr. Hbz_{ins}	A	−	−	−	−	±	+	+	+	++			
	B	+	++	++	++	++	++	++	++	++			
Div. I-Fr. Cbz_{ins}	A	−	−	−	−	−	−	−	±	+	+		
	B	+	+	+	++	++	++	++	++	+++			
Div. I-Fr. Cbz_{sol}	A	−	−	−	−	−	−	−	−	−	−	+	
	B	+	+	++	++	++	++	++	++	++	+++		
Div. II-Fr. Et_{ins}	A				−	−	−	−	−	−	−	−	±
	B				+++	+++	+++	+++	+++	+++	+++	+++	+++

2) Solubility.
See Table II.

3) Composition.
The final fraction of Div. I, which had not come out of benzene when cold, analyzed: N 3.2%, P 2.4%, total carbohydrate as glucose 10.7%, ash 11.1%.

II. Group-active Fractions of the Lipids from Submucosa of Pig Stomachs.

The dehydrated material (128 g.) was subjected to fractionation as above, but the process was modified or stopped halfway because of low

TABLE II.

Solubilities of the Group-active Lipid Fractions of Mucosa propriae together with Surface Epithelium.

Fractions are named as in the text.

−: insoluble, +: partly sol., ++: mostly sol., +++: completely sol.

Fraction	Solvent															
	Water		Abs. alc.		Acetone		Ether		Petrol ether		Benzene		Chloro-form		Tetralin	
	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot
Div. I-Fr. Cbz_{ins}	++	++	−	+	−	−	−	−	−	−	−	+++	+++	+++	−	+++
Div. I-Fr. Cbz_{sol}	++	++	−	+	−	−	−	−	−	−	++	+++	+++	+++	−	+++
Div. II-Fr. Et_{ins}	+++	+++	−	+			−	−	−	−	+++	+++	+++	+++	−	+++

activity or small yield of intermediate products.

The deposits from the hot-alcoholic tissue-extracts (930 cc.) when cold —**Div. II'**—(white and waxy) amounted to 6.5 g.

The mother liquid left a syrupy residue with brown shade on distillation—**Div. I'**. After thoroughly washing it with acetone and drying there remained 1.8 g. of a brown hard clump. This fraction was subdivided with cold abs. alcohol as the corresponding fraction in Div. I. The condensation of the alcoholic solution and treatment of the remainder with abs. alcohol after washing with acetone was repeated twice. The insoluble portions were 0.5 g. altogether—**Fr. Alc_{ins}** (a yellowish powder). The third alcoholic solution was distilled and the residue here (**Fr. Alc_{sol}**, a brown gelatine-like substance weighing 1.2 g.) and Fr. Alc_{ins} were completely dried and further treated with ether separately. They both did not dissolve at all. After this operation Fr. Alc_{ins} was boiled with benzene but found again insoluble, while assay of Fr. Alc_{sol} showed it somewhat soluble in this solvent. Their serological potencies are illustrated in Table III.

Div. II' was diminished to 5.2 g. by washing with acetone. A white fine powder thereby given dissolved mostly (5.1 g.) in dry ether at the next stage. The insoluble part (0.1 g.) was inhibitory to the iso-agglutination of A erythrocytes only at a dilution of 1:100. Therefore the soluble part which was twice active and more in amount was subdivided by the aid of cold abs. alcohol (30 cc.). Of 4.9 g. of the fraction 4.8 g. were left undissolved and the evaporated residue of the solution amounted to 0.1 g. —**Fr. Alc_{sol}**. The former was inactive at a dilution of 1:100. The latter displayed the action at the dilution 1:400, but was insufficient in quantity for further fractionation. Its solubilities are shown in Table IV.

TABLE III.

The Potency inhibitory to Iso-agglutination of A Erythrocytes of Div. I'-Fr. Alc_{sol} and -Fr. Alc_{ins} from Submucosa.

B serum had agglutinin titre 320 and was diluted to 1:20.
—: no agglutination, ±: faint aggl., +: slight aggl.

Fraction	Dilution of substances				
	$\times 1 \times 10^2$	$\times 2 \times 10^2$	$\times 4 \times 10^2$	$\times 8 \times 10^2$	$\times 1.6 \times 10^3$
Alc_{sol}	—	—	+	+	+
Alc_{ins}	—	±	+	+	+

TABLE IV.

Solubilities of Div. II'-Fr. Alc_{sol} from Submucosa.

—: insoluble, ##: completely sol.

Solvent	Water		Abs. alc.		Acetone		Ether		Benzene		Chloroform		Tetralin	
	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot
Grade of dissolution	—	—	—	##	—	—	##	##	##	##	##	##	##	##

Through the Grant Committee of the Science Council was given a grant from the Education Department which enabled us to carry out this work.

H. Masamune.

References.

1) Masukawa, Tohoku J. Exp. Med., 1951, **54**, 227.
2) Masamune and Siozima, ibid., this volume, p. 319.

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A Method of the Colorimetric Determination of Noradrenaline and Adrenaline by Employing the Permanganate Reagent.

By

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A method for the differential estimation of noradrenaline and adrenaline using the iodine reaction was reported by Euler and Hamberg (Acta Physiol. Scand., 1949, **19**, 74). However in practice it has an inconvenience, as the fact the tint after the oxidation with iodine is not uniform, which was mentioned in the paper of Euler and Hamberg themselves.

In our method, the permanganate reaction is employed instead of iodine reaction and the identical tint after oxidation can always be obtained. Adrenaline and noradrenaline are oxidized by the permanganate reagent at pH 3.6 and at pH 5.6 respectively, with the different oxidation rate. At pH 3.6, adrenaline is oxidized completely within 2 minutes and only less than 10% of noradrenaline is oxidized during the same time. On the contrary, at pH 5.6, adrenaline as well as noradrenaline can be oxideized fully within 3 minutes.

The procedure of our method is as follows. 1 cc. of a sample for estimation or a standard is measured, which after pre-adjusting, if necessary, to the neighbourhood of pH 3.6 and 5.6 respectively, is adjusted to pH 3.6 and 5.6 by using n-acetate-acetic acid buffer solutions. Then 0.1 cc. of the permanganate reagent (3 g. potassium permanganate, 24 cc. distilled water, 8 cc. lactic acid) is added. After 2 minutes at pH 3.6 or 3 minutes at pH 5.6 0.1 cc. of 3% hydrogen peroxide is added and the mixture is diluted with water up to the volume of 6 cc. Comparison of colour density is then performed.

The amounts of noradrenaline and adrenaline can be calculated from the following equations.

$$A = x + n_{3.6} \cdot y, \qquad B = x + n_{5.6} \cdot y.$$

The symbols have the following meaning; x=the amount of adrenaline, y=the amount of noradrenaline, A=adrenaline equivalent estimated at pH 3.6, B=the same estimated at pH 5.6, $n_{3.6}$=the ratio of doses (adrenaline: noradrenaline), necessary to form the same colour density after oxidation at pH 3.6 in 2 minutes, $n_{5.6}$=the same at pH 5.6 in 3 minutes.

We were able to apply this method successfully to the extract of bovine suprarenal medulla. The details will be reported by one of us (O.) in this journal.

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Blood Group Lipoids from Human Gastric Mucosa and Gastric Cancer.*

By

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This investigation was undertaken to compare, regarding the composition, not only A, B and O group lipoid of the gastric mucosa with one another but also one of the corresponding lipoids of gastric cancer with them. The tissue lipids extractible with hot alcohol were fractioned in a similar manner as already reported,[1] but the final stage by means of hot alcohol was not processed due to lack of material excepting when those from the mucosa of stomachs of Group A persons were dealt with. In this case, however, there was given no subdivision allusive to a pure state of the lipoid. Despite of the probable crudeness, the preparations (The subdivisions of the Group A lipoid by the final stage operation are excluded) of the different group lipoids gave nearly concordant figures: N 3.6–3.9%, P 2.6–2.8%, total carbohydrate as glucose 9.4–11.9%, ash 7.8–12.4% (3.9–4.3% N, 2.8–3.1% P and 10.3–13.1% total carbohydrate on an ash-free basis). The equivalent ratios of N to P, 2.9–3.1 : 1. On these analyses, the writer inclines to believe that the different blood group lipoids of gastric mucosa and gastric cancer resemble all one another or rather identical in so far as the composition concerns.

Of the lipoids from the normal mucosa, the Group A substance was anti-iso-hemoagglutinative at a dilution of $1:2.56\times10^4$–5.12×10^4 and the Group B substance at a dilution of $1:2.56\times10^4$, and the Group O substance inhibited the agglutination of O erythrocytes by an anti-O immune serum treated in advance with Be erythrocytes at a dilution of $1:1.28\times10^4$. The Group A substance from gastric cancer was specifically active at a dilution of $1:6.4\times10^3$. That the group lipoid from gastric cancer is distinctly less active than the corresponding substance of the normal mucosa reminds the writer of the weak activity of the group lipoid which

* 8th report of Masamune and co-workers' "Chemistry and Biology of Lipids."

was separated by Aikawa[2] from a metastasis of gastric cancer in a person belonging to Group B and had like contents of N (3.6%) and P (2.6%) (ash, 8.10%) as above. (The author applied indole, orcinol and diphenylamine methods immediately to his preparations for sugar determination, but the values obtained are much larger than the actual as Matuda[3] mentioned and, in addition, what they signify is obscure.)

Judging from the analyses of the group lipoids (concentrates) of erythrocytes (Hamasato[4]) and of liver (Matuda[3]) as well as the finding above, the group lipoids involve N and P in a ratio characteristic to their source. Hence the N:P ratio of the group lipoid in gastric cancer furnishes a proof that gastric cancer has a group lipoid which is peculiar to its site and differ from the one in the normal mucosa only in the grade of differentiation. And, moreover, it is most plausibly assumed that Aikawa's lipoid from the metastasis and the corresponding lipoid in the original growth are identical just like the group carbohydrates of those primary and secondary tumors (Cf. Oh-Uti[5] and Matuda[6]).

What kind of change in structure or molecular form or in some other respect accompanies the biological differentiation must be a very important problem awaiting solution.

Experimental.

1) The Group Lipoid from the Mucosa of Stomachs of Persons Belonging to Group A.

The mucosa torn from muscularis externa was freed mechanically from mucus and minced. 1254 g. of the fresh material thus obtained from 19 stomachs were dehydrated by keeping under 4 volumes of alcohol for many days. 224 g. was the material dried. It was boiled twice with abs. alcohol (5 and 3.5 volumes were used). The extracts were 1800 cc. altogether. After standing overnight the sticky deposits were filtered off and set aside—**Div. II** (1.5 g.). The filtrate (light brown and transparent) was distilled in vacuo and the semifluid residue—**Div. I**—was covered under 100 cc. of acetone for hours. The acetone-insoluble part was separated and after washing with changes of acetone until no colored matter came out, placed in a desiccator. 4.5 g. of a brownish clayey substance (hygroscopic) were yielded. From another batch of tissue material (481 g. in fresh state, 68 g. when dehydrated) was given 0.8 g. of the division corresponding to Div. II above—**Div. II'**—and 1.2 g. of the acetone-insoluble part of the division corresponding to Div. I above (**Div. I'**).

The balances of the acetone-insoluble parts of Divs. I and I', after serological and other assays, were united (total weight 5.5 g., anti-isohemoagglutinative at a dilution of 1:3200), exhaustively treated with cold abs. alcohol (Five 30 cc. portions of abs. alcohol were used; the final change

of alcohol remained uncolored). The extracts were distilled together to dryness and placed in a desiccator. Since the distilled remainder was found pretty sero-reactive (active at a dilution of 1:1600), complete separation from it of the part, which involves the group lipoid and is insoluble per se, was tried by repetitions of a similar procedure. All the residues, dried and put together, (3.6 g. in total; the sero-reactive dilution, 1:6400) were washed with acetone, dried and subjected to ether fractionation, namely, they were agitated with 36 cc. of dry ether and centrifuged and the supernatant fluid was distilled in vacuo. The centrifugate (1.7 g.) and the distilled residue here (1.0 g.) possessed nearly equal serological potencies (The dilutions where they were active were 1:12800 and 1:6400 respectively). However, on another treatment of the latter with dry ether, after thorough drying (in a vacuum desiccator for 3 days), the part that remained undissolved (0.3 g.) was found active even at a dilution of 1:32,000, whereas the one which had been recovered from the solution (0.7 g.) was active merely at a dilution of 1:1600. Followingly the both insoluble parts (2.0 g. in total) were refluxed with 20 cc. benzene on a water-bath again and again (five times). Each time the mixture was centrifuged while hot. The supernatant fluids were distilled in vacuo. The amorphous brown gelatine-like substance left (**Prep. I**, 1.2 g.) exhibited the specific anti-iso-hemoagglutinative potency up to 5.12×10^4 times dilution (s. Table I).

TABLE I.

Anti-iso-hemoagglutinative Potency of Prep. I from the Mucosa of Stomachs of Persons Belonging to Group A.

Both B and A serum had agglutinin titre 160 and were diluted to 1:10.
−: no agglutination, ±: faint aggl., +: slight aggl.,
++: marked aggl., ###: strong aggl.

Group of erythroc.	Dilution of the substance											
	$\times\frac{1}{10^3}$	$\times 10^2$	$\times 10^2$	$\times 10^2$	$\times 10^3$	$\times 10^3$	$\times 10^3$	$\times 1.28 \times 10^4$	$\times 2.56 \times 10^4$	$\times 5.12 \times 10^4$	$\times 1.024 \times 10^5$	$\times 2.048 \times 10^5$
A	−	−	−	−	−	−	−	−	−	−	+	+
B	−	+	+	+	++	++	++	++	++	###	###	###

The final product had solubilities in Table II. Its analyses and optical rotation are shown in Table III.

The substance was considered a mixture when analogized from the finding on the corresponding product from pig gastric mucosa. Hence, a further fractionation by the aid of abs. alcohol was made, but no definite knowledge could be acquired as to the nature of the pure group lipoid.

TABLE II.

Solubilities of Prep. I from the Mucosa of Stomachs of Persons Belonging to Group A.

−: insoluble, ±: faintly sol., +: partly sol., ++: mostly sol., +++: completely sol.

Solvent	Water cold	Water hot	Abs. alcohol cold	Abs. alcohol hot	Acetone cold	Acetone hot	Ether cold	Ether hot	Petrol ether cold	Petrol ether hot	Benzene cold	Benzene hot	Chloroform cold	Chloroform hot	Tetralin cold	Tetralin hot	Glacial acetic acid cold	Glacial acetic acid hot
Solubility	+++	+++	−	+	−	−	−	−	−	−	+++	+++	+++	+++	−	+++	−	−

TABLE III.

Composition and Optical Rotation of Prep. I from the Mucosa of Stomachs of Persons Belonging to Group A.

Analysis	In per cent	In equivalents per equivalent weight
N	3.9	3.1
P	2.8	1.0
Total carbohydrate as glucose*	11.9	
Ash†	8.9	

* Masamune and Tanabe procedure[7]. Since no phosphotungstic acid-precipitable substance was found in the filtrate after hydrolysis, this precipitant was not employed, neutralizing immediately the filtrate for the determination of the reducing force. † Micro analysis.

$$[\alpha]_D^{19}=\frac{+0.023\times 100}{1.236\times 1}=+1.9^\circ \text{ (in chloroform)}.$$

The operation was effected as follows:—200 mg. of the substance were treated with hot abs. alcohol again and again (five times) by refluxing each time with a 15 cc. portion of the solvent for 20 minutes. The extracts were decanted out, while hot. The brown insoluble part that had adhered to the vessel wall was semifluid while hot, and solidified on cooling —**Fr. I** (30 mg.). The extracts were combined and stood in an ice-chest for one day, whereupon coarse flocculent precipitates (white) appeared. These precipitates were centrifuged and dried—**Fr. II** (a white powder weighing 80 mg.). The supernatant fluid was evaporated to about 10 cc. and again placed in an ice-chest. The occurring deposits were also separated after one day and dried—**Fr. III** (a white powder, 30 mg.)—and the mother liquid was distilled in vacuo to dryness—**Fr. IV** (a yellow powder, 35 mg.). The anti-iso-hemoagglutinative forces and analyses of the fractions are shown in Tables IV and V.

To Divs. II and II' united (2.3 g.) was adapted the above series of fractionation. a) At first they were washed well with 20 cc. portions of

TABLE IV.

Anti-iso-hemoagglutinative Potency of the Fractions of Prep. I from the Mucosa of Stomachs of Persons Belonging to Group A.

Fractions are named as in the text. The B serum used had agglutinin titre 320 and was diluted to 1:20.
—: no agglutination, ±: faint aggl., +: slight aggl., ++: marked aggl.

Fraction	Group of erythroc.	Dilution of substances								
		×4 ×10²	×8 ×10²	×1.6 ×10³	×3.2 ×10³	×6.4 ×10³	×1.28 ×10⁴	×2.56 ×10⁴	×5.12 ×10⁴	×1.024 ×10⁵
I	A	—	—	—	—	±	+	++	++	++
II	„	—	—	—	—	—	—	—	±	+
III	„	—	—	—	—	—	—	—	—	±
IV	„	—	—	—	—	±	+	++	++	++

TABLE V.

Composition of the Fractions of Prep. I from the Mucosa of Stomachs of Persons Belonging to Group A.

Fractions are named as in the text. Analyses are expressed as percentages.

Fraction	Analysis			
	N	P	Total carbohydrate as glucose*	Ash†
I	5.0	2.5	16.0	12.5
II	3.2	1.3	14.6	5.6
III	3.7	1.9	13.0	3.7
IV	4.4	3.6	8.3	9.6

* Calculated from the reducing force of the hydrolysate. Analysis was made as described in the Note of Table III. † Micro analysis.

dry acetone and dried. A hygroscopic brownish powder otbained was sero-reactive at a dilution of 1:8000, and amounted to 0.45 g. b) This was agitated twice with 20 cc. abs. alcohol, each time with following centrifugation. The insoluble part (a hygroscopic white powder) was 150 mg. The supernatant fluids (brownish and transparent) were distilled and the residual, washed with acetone (20 cc.) and dried, (a brownish stuff matter) was again treated with abs. alcohol as above. 100 mg. was the insoluble part here. The substance recovered from the solution (180 mg.) was non-active. c) The parts undissolved (sero-reactive at a dilution of 1:16000) were again washed with acetone and completely dried, and then washed with changes (three 30 cc. portions) of ether. Almost no loss of weight occurred, but the substance (a hygroscopic, yellowish, fine powder) became twice as active as the product in b). d) It was boiled with 20 cc. benzene

under return condenser for 20 minutes and centrifuged when cold, and the centrifugate was treated likewise once again. The extracts were distilled to dryness. 100 mg. of a gelatine-like substance with brown shade were given. This had the same potency as the material before the fractionation, notwithstanding that the benzene-insoluble part (a white powder amounting to about 100 mg.) was almost inactive (anti-iso-hemoagglutinative at a dilution of 1:400). The serological potency, analyses and solubilities of the final product (**Prep. II**) are illustrated in Tables VI, VII and VIII.

TABLE VI.

Anti-iso-hemoagglutinative Potency of Prep. II from the Mucosa of Stomachs of Persons Belonging to Group A.

B and A serum had agglutinin titres of 320 and of 160 and were diluted 20 and 10 times respectively.

−: no agglutination, ±: faint aggl., +: slight aggl., ++: marked aggl., ###: strong aggl.

Group of erythrocytes	Dilution of the substance										
	×1 ×10^2	×2 ×10^2	×4 ×10^2	×8 ×10^2	×1.6 ×10^3	×3.2 ×10^3	×6.4 ×10^3	×1.28 ×10^4	×2.56 ×10^4	×5.12 ×10^4	×1.024 ×10^5
A	−	−	−	−	−	−	−	−	−	±	+
B	−	+	++	++	++	++	++	###	###	###	###

TABLE VII.

Composition of Prep. II from the Mucosa of Stomachs of Persons Belonging to Group A.

Analysis	In per cent	In equivalents per equivalent weight
N	3.6	3.1
P	2.6	1.0
Total carbohydrate as glucose*	11.0	
Ash†	7.8	

*, † Signify the same as in Tables III and V.

TABLE VIII.

Solubilities of Prep. II from the Mucosa of Stomachs of Persons Belonging to Group A.

−: insoluble, +: partly sol., ++: mostly sol., ###: completely sol.

Solvent	Water		Abs. alcohol		Acetone		Ether		Petrol ether		Benzene		Chloro-form		Tetralin		Glacial acetic acid	
	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot
Solubility	++	###	−	+	−	−	−	−	−	−	++	###	++	###	−	###	−	++

2) The Group Lipoid from the Mucosa of Stomachs of Persons Belonging to Group B.

The fresh mucosa amounted to 839 g. (13 stomachs) altogether and the dehydrated to 156 g. The hot-alcoholic extracts were 1200 cc. in total.

The precipitates (**Div. II**, 100 mg.) from the tissue extracts when cold were washed with acetone. The substance after the washing (70 mg.; mostly soluble in water) exhibited anti-iso-agglutinative force towards B erythrocytes at a dilution of 1:6400. However, because of small quantity of the material, the group lipoid in it was not traced.

The remainder after distillation of the mother liquid of Div. II—**Div. I**—was treated with acetone as in 1), and 1.2 g. of a coarse brownish powder (hygroscopic) were given. Further fractionation was carried out also similar to above, and 0.65 g. of the cold-alcohol-insoluble substance (a yellowish powder), 0.42 g. of the ether-insoluble (a fine white powder) and 0.12 g. of the hot-benzene-soluble (a fine white powder) were obtained in turn. The final product inhibited the iso-agglutination of B erythrocytes at a dilution of $1:2.56\times10^4$ and that of A erythrocytes at a dilution of $1:4\times10^2$, and had the same solubilities as Prep. I of the Goup A lipoid above. Analayses: N 3.6%; P 2.6%; total carbohydrate as glucose 9.4%; ash 8.3%. $[\alpha]_D^{15}=\frac{+0.021\times100}{1.023\times1}=+2.1°$ (in chloroform).

3) The Group Lipoid from the Mucosa of Stomachs of Persons Belonging to Group O.

In this case, the deposits from the hot-alcoholic tissue extracts when cold and the substance that remained in solution were fractioned without serological assay of the intermediate products. The final product from the former division exhibited remarkable reaction with an anti-O serum treated with Be erythrocytes, while that of the latter was entirely devoid of the activity.

The fresh mucosa, 927 g. (number of stomachs, 14). The tissue after dehydration, 174 g. The hot-alcoholic extracts altogether, 1260 cc. The deposits in the cold were separated and dried and 0.7 g. of a yellowish brittle clump was given—**Div. II.** From the distilled residue (a syrup) of the mother liquid—**Div. I,** 1 g. of the acetone-insoluble substance (a coarse brownish hygroscopic powder), 0.6 g. of the cold-abs. alcohol-insoluble, 0.45 g. of the dry-ether-insoluble and 0.35 g. of the hot-henzene-soluble (a light, brown powder) were obtained in turn. Yields of the products by successive subdivision of Div. II were: the acetone-insoluble (a coarse hygroscopic powder with brown shade), 0.5 g.; the cold-abs. alcohol-insoluble, 0.4 g.; the dry-ether-insoluble (a fine, very hygroscopic,

white powder), 0.35 g.; the hot-benzene-soluble (a brown gelatinous substance), 0.25 g.

The both final products showed solubilities similar not only mutually but also to the corresponding preparations of the Group A and B lipoids. Each of them was Group A-active at a dilution of 1:400 and Group B-active at a dilution of 1:200, but the substance produced from Div. II alone reacted with an anti-O serum [Increasig volumes (8, 10, 12, 14, 16 and 18 cc.) of 10% suspensions of O erythrocytes in physiological saline were injected at intervals of two days into V. jugularis communis of a goat, and after 1 week from the last injection, the blood was shed] adsorbed with Be erythrocytes at a dilution of $1:1.28\times10^4$ (s. Tables IX and X).

TABLE IX.

Agglutination of O and Be Erythrocytes by an Anti-O Serum Treated with Be Erythrocytes.

Adsorption: To 3 cc. of the immune serum diluted 10 times was added 1 cc. of Be erythrocytes and after standing at room temp. (8°C) for 30 minutes, centrifuged.

−: no agglutination, ±: faint aggl., +: slight aggl.

Group of erythrocytes	Dilution of the immune serum				
	×10	×20	×40	×80	×160
O	+	+	+	±	−
Be	±	−	−	−	−

TABLE X.

Agglutination of O Erythrocytes by an Adsorbed Anti-serum, which had been Treated in Advance with the Final Products from the Mucosa of Stomachs of Persons Belonging to Group O.

The serum diluted 10 times and treated with Be erythrocytes in Table IX was employed for the present assay.

−: no agglutination, ±: faint aggl., +: slight aggl.

Division wherefrom the substance was obtained	Dilution of substances								
	×1 ×10²	×2 ×10²	×4 ×10²	×8 ×10²	×1.6 ×10³	×3.2 ×10³	×6.4 ×10³	×1.28 ×10⁴	×2.56 ×10⁴
I	±	+	+	+	+	+	+	+	+
II	−	−	−	−	−	−	−	−	+

The Group O-active product analyzed: N 3.6%; P 2.7%; total carbohydrate as glucose 9.4%; ash 11.3%. It had the rotation: $[\alpha]_D=\frac{+0.015\times100}{1.011\times1}=+1.5°$(in chloroform). Its solubilities resembled

those of the corresponding product of the Group A lipoid.

4) The Group Lipoid from Gastric Cancers of Persons Belonging to Group A.

Cancer material: 632 g. (30 cancers) in a fresh state and 98.5 g. when dehydrated.

The deposits, that had come out from the hot-alcoholic tissue extracts when cold, were no more than 50 mg. and, in addition, their part insoluble in acetone (30 mg.) inhibited the iso-agglutination of A erythrocytes only at a dilution of 1:100. The transparent yellowish mother fluid was distilled in vacuo and the remainder treated with acetone as above. The insoluble part here amounted to 0.5 g. The cold-alcohol-insoluble substance separated therefrom was 0.36 g. and on fractioning with ether of its balance after serological assay (0.35 g.) remained 0.3 g. of a fine white powder undissolved. Of this 0.25 g. dissolved in hot benzene. The preparation finally obtained was a brownish brittle clump and exhibited the specific inhibitory power to iso-agglutination of A erythrocytes at a dilution of 1:6400. It had solubilities same as the corresponding preparations from normal gastric mucosa. N 3.6%, P 2.6%, total carbohydrate as glucose 10.9%, ash 12.4%. $[\alpha]_D^{28}=\frac{+0.040\times 100}{1.028\times 1}=+3.9°$(in chloroform).

Summary.

1) It was tried to separate the blood group lipoids by the aid of organic solvents from human gastric mucosa and gastric cancers.

2) All of the preparations of the A, B and O group-lipoids had similar composition with respect to N-, P- and carbohydrate-content and similar solubilities.

3) They rotated D light to the right but slightly.

4) Group A lipoid from gastric cancer was serologically much less active than the corresponding lipoid from the normal mucosa.

5) An assumption was expressed that the liver metastasis of gastric cancer contains a group lipoid same as the one in its original growth.

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Adrenaline Secretion of the Suprarenal Glands in Cyanide Anoxia.*

By

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The references concerning the adrenaline secretion of the suprarenal glands in cyanide anoxia are few in number, although some experimentalists demonstrated that the exclusion of the suprarenals is capable of reducing or abolishing the effect of cyanide on the blood sugar or on the blood pressure.

In a suprareno-jugular anastomosis experiment, Houssay and Mollnelli[1] injected 30 mg potassium cyanide intravenously in the donor dog (31 kg.). One and a half minutes after injection, the elevation of blood pressure of the recipient dog (29 kg.) from 120 mm. Hg to 234 mm. Hg was observed. The rise of blood pressure in the donor dog also took place, but in an extent not so much remarkable as in the recipient dog.

Employing the perfused toad hind limb preparation for the estimation of adrenaline, Kudo[2] measured the adrenaline content in the peripheral arterial blood of rabbits in cyanide poisoning. Although his results were not conclusive, the increase of adrenaline content in the peripheral arterial blood to 1:1,000,000–1:10,000,000 was estimated.

On perfusing the excised suprarenal gland, Ssentjurin[3] was unable to find any inhibitory action of cyanide salt on the adrenaline secretion of suprarenals and on the excitability of the suprarenals.

Now some references, which have a close relation to the present problem and in which the adrenaline secretion in cyanide poisoning was studied indirectly, will be quoted as follows.

Evans[4] noticed in his studies that the elevation of blood pressure caused by a small dose of NaCN becomes smaller in magnitude after the exclusion of the suprarenal glands from the circulation. He assumed from this result that the elevation of blood pressure in cyanide poisoning

* Reported at the XXIII Annual Meeting of the Japanese Physiological Society at Sendai, Nov. 1946; Nihon Seiri Z., 1947, **10**, 283.

may partly be due to the accelerated adrenaline secretion.

Regarding the cyanide hyperglycemia and the suprarenal gland, Kudo[2] observed that the increase of blood sugar after cyanide injection in the rabbits doubly suprarenalectomized is far smaller in the magnitude in comparison with that in the normal rabbits.

On the other hand, Ohmi[5] in our laboratory was able to see a remarkable increase of blood sugar also in doubly suprarenalectomized rabbits with a magnitude of a little inferior to that in the normal rabbits. He concluded in his report that the suprarenal glands play a rather small share in causing the cyanide hyperglycemia.

Some years after, Møller and Stefansson[6] did not observe the cyanide hyperglycaemia in rabbits after removing the suprarenal glands, while in normal rabbits a definitely increased blood sugar was noticed after cyanide injection. They assumed from this result that the cyanide hyperglycaemia is wholly due to the accelerated adrenaline secretion of the suprarenal gland.

Purposing to know quantitatively and precisely the influence of cyanide anoxia upon the adrenaline secretion of the suprarenal glands in animals under wholly physiological condition as possible, the present investigations were undertaken.

Methods.

The present investigations were carried out on six dogs, in which D_{11}-L_3 dorsal spinal roots were sectioned about four weeks or more previous to the experiment.

As usually the suprarenal vein was exposed and the blood samples were collected therefrom without anesthesia.[7] The estimation of adrenaline content in the suprarenal vein blood specimens was performed by using the rabbit intestine segment method. The arterial bood pressure tracing was registered by using the Hg-manometer connected with the femoral artery. The blood sugar content was estimated by Hagedorn and Jensen's method.

Potassium cyanide ("Merck") was as a 0.5% or 1.0% solution injected intravenously in a dose of 1.0,1.25,2.0 and 2.5 mg. per kg. of body weight in thirty seconds, or injected subcutaneously in a dose of 1.5,3.0 and 4.0 mg. per kg.

Results.

In Exp. 1 potassium cyanide was injected intravenously in a dose of 1.0 mg. per kg. Soon after the start of injection the respiratory movements became deep and frequent and just after the end of injection covulsions occurred on the whole body. The nausea and vomiting took place. The blood pressure elevated from 89 mm. Hg to 117 mm. Hg. The rate of heart beat increased. The rate of adrenaline secretion from one gland

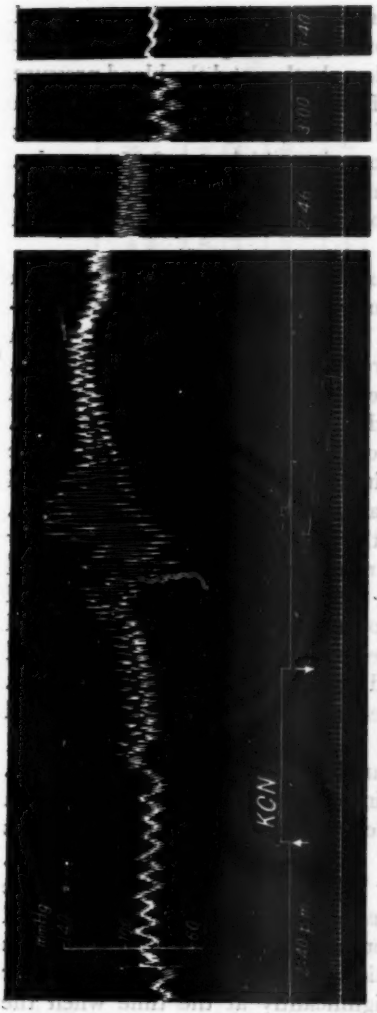

Fig. 1. Blood pressure tracings in Exp. 2. (Reduced to 1/2) Dog 11.2 kg. ♂, non-anesthetized. KCN (1.25 mg. per kg. of body weight) was injected intravenously. Time in seconds.

was 0.01–0.02 γ per kg. per minute before injection. After cyanide injection, it increased step by step and reached 0.62 γ per kg. per minut one and a half minutes after the injection. The samples taken six minutes or more after injection did not show any acceleration of adrenaline secretion. The blood sugar increased slightly (from 0.101% to 0.134%), but soon it returned to the initial value.

1.25 mg. per kg. potassium cyanide was injected intravenously in Exp. 2. After injection deep respiration was observed. The animal stretched his limbs. The blood pressure rose from 86 mm. Hg gradually and reached 130 mm. Hg about one and a half minutes after the start of injection. The heart rate increased definitely. Then the blood pressure descended gradually. The initial rate of adrenaline secretion was 0.02-0.03 γ per kg. per minute. It was found to increase in the first sixty-second-period to 0.23 γ per kg. per minute. The specimens taken in the second and third sixty-second-periods showed a slightly accelerated adrenaline secretion such as 0.06 γ and 0.09 γ per kg. per minute. The blood sugar content measured as indicating a slight increase (from 0.108% to 0.125%) in 6 minutes and 8 minutes after the injection. Thereafter it decreased very slowly and resumed almost its initial value 30 minutes after injection.

In Exp. 3, 2.0 mg. potassium cyanide per kg. was injected intravenously in 30 seconds. During injection the respiratory movements became intensiver and the convulsions on the whole body was observed. About

10 seconds after the start of cyanide injection the heart beat stopped suddenly and the blood pressure fell from 92 mm. Hg to 20 mm. Hg, but soon the heart commenced to beat with slow rhythm and the blood pressure ascended again and reached 120 mm. Hg at the end of injection. Then the respiratory movement stood still and the animal died about 7 minutes after injection. The adrenaline secretion rate which was 0.05 γ per kg. per minute before injection increased during the injection to 0.3 γ per kg. per minute. Just after the end of injection it increased further and reached 1.9 γ per kg. per minute.

Similar results were derived from Exp. 4, in which 2.5 mg. potassium cyanide per kg. was injected intravenously. During injection the respiration became deep and the convulsion set in on the whole body, which lasted about 15 seconds. The blood pressure rose at first from 110 mm. Hg and reached 136 mm. Hg. Soon after the end of injection it descended promptly to 18 mm. Hg and again ascended. Now the respiration stopped. The slowing of the heart was observed concomitantly with the fall in the blood pressure. The animal died 5 minutes after injection. The initial rate of adrenaline secretion was in this case 0.06–0.07 γ per kg. per minute. While the specimen taken in the first thirty-second-period did not show any alteration of adrenaline secretion rate, an enormously accelerated rate of adrenaline secretion such as 1.9 γ per kg. per minute was estimated with the next specimen, the collection of which started just after the end of injection and lasted 30 seconds. The next specimen taken in the third 30 seconds showed somewhat increased secretion such as 0.29 γ per kg. per minute. The highest level of adrenaline secretion in Exp. III and Exp. IV was therefore just the same.

In other two dogs, potassium cyanide was injected subcutaneously. 1.5 mg. potassium cyanide per kg. was injected in Exp. 5. After injection the animal shivered. About 25 minutes after injection both hind limbs were stretched. 10 minutes thereafter tonic convulsions occurred twice. The heart rate increased slightly after injection and the blood pressure descended very slowly and slightly. The blood sugar content was measured to decrease from 0.111% slightly after injection. Its lowest level was 0.085%. The rate of adrenaline secretion, which was 0.02 γ per kg. per minute before injection, increased insignificantly at the time when the convulsions were observed.

In Exp. 6, potassium cyanide was injected subcutaneously at first in a dose of 3.0 mg. per kg. Except a salivation no symptoms of cyanide poisoning were observed. The blood pressure descended gradually and the heart rate increased slightly. One hour after the first injection 4.0 mg. potassium cyanide was injected subcutaneously. About half an hour after the second injection the animal became restless and the deep respiration

TABLE I.

Adrenaline Secretion of the Suprarenal Gland, Blood Sugar, etc. after Injection of Potassium Cyanide.

Time	Time after KCN injection (sec.)	No. of specimen	Quantity of blood (cc.)	Duration of collection (sec.)	Blood flow per kg. per min. (cc.)	Adrenaline in 1 cc. blood (γ)	Adrenaline secretion per kg. per min. (γ)	Blood sugar (%)	Blood pressure (mm. Hg)	Heart rate (per min.)	Respiration (per min.)
Exp. 1. 18. IV. 1944 Dog ♂ 15.6 kg.											
17. III. 1944 D_{II}-L_3 dorsal spinal roots sectioned. (16.9 kg.)											
10. 15 a. m.–0.20 p. m. Left lumbar route preparation. Left femoral artery and right saphena vein prepared.											
3: 15 p.m.		1	2.2	30	0.28	0.05	0.01	0.101	93	126	9
		2	2.3	30	0.30						
3: 25		3	2.8	30	0.36	0.05	0.02	0.101	89	129	14
3: 40	KCN (1.0 mg. per kg.) was injected intravenously in 30 seconds.										
	0– 30	4	2.9	30	0.37	0.05	0.02		89	132	15–42
	30– 60	5	3.1	30	0.40	0.1	0.04		89–104– 84	150	48
	60– 90	6	3.1	30	0.40	0.3	0.12		84–117– 86	168	27
	90–120	7	2.4	30	0.31	2.0	0.62	0.106	86– 96	120	30
3: 46		8	1.8	30	0.23	0.05	0.01	0.134	93	129	15
3: 55		9	2.6	30	0.33	0.075	0.02	0.104	86	138	12
		10	2.3	30	0.30						
4: 10		11	2.4	30	0.31	0.05	0.02	0.090	88	168	12
		12	2.3	30	0.30						
4: 25		13	2.5	30	0.32	0.1	0.03	0.095	82	180	8
		14	2.4	30	0.31						
4: 40		15	2.0	30	0.26	0.05	0.01	0.088	82	168	12
		16	1.9	30	0.24						
Room temperature 16–17°C.											
Body temperature 38.5–39.2°C.											
Exp. 2. 26. IV. 1944 Dog ♂ 11.2 kg.											
24. III. 1944 D_{II}-L_3 dorsal spinal roots sectioned. (11.9 kg.)											
10: 10 a. m.–11.55 a.m. Left lumbar route preparation. Left femoral artery and right saphena vein prepared.											
2: 20 p.m.		1	2.7	30	0.48	0.05	0.02	0.102	78	126	15
		2	2.8	30	0.50						
2: 30		3	3.0	30	0.54	0.05	0.03	0.108	78	138	15
2: 40	KCN (1.25 mg. per kg.) was injected intravenously in 30 seconds.										

	0– 60	4	6.5	60	0.58	0.4	0.23		86–119	162	18–57
	60–120	5	4.8	60	0.43	0.15	0.06		119–130–114	234–162	48
	120–180	6	3.5	60	0.31	0.3	0.09		114–102	162–126	39
	180–240	7	4.5	60	0.40	0.1	0.04		102	120	36
2: 46		8	2.0	30	0.36	0.1	0.04	0.125	97	111	36
2: 50		9	2.2	30	0.39	0.1	0.04	0.125	84	120	24
2: 55								0.124	78	132	14
3: 00								0.119	76	156	18
3: 10		10	2.1	30	0.38	0.05	0.02	0.108	76	180	15
3; 20								0.099	72	174	17
3: 30								0.099			
3: 40		11	1.9	30	0.34	0.05	0.02	0.108	84	186	15

Room temperature 17–19°C.
Body temperature 38.4–39.2°C.

Exp. 3. 25. II. 1944 Dog ♂ 9.8 kg.

29. XII. 1943 D_{11}-L_3 dorsal spinal roots sectioned. (11.3 kg.)

10: 25 a.m.—11: 40 a.m. Left lumbar route preparation. Left femoral artery and right saphena vein prepared.

2: 40	p.m.	1	2.4	30	0.49	0.1	0.05	0.094	95	150	21
		2	2.3	30	0.47						
2: 55		3	2.3	30	0.47	0.1	0.05	0.099	95	150	20

3: 12 KCN (2.0 mg per kg) was injected intravenously in 30 seconds.

0– 30	4	1.8	30	0.37	0.8	0.30		92– 20–120	150–0–54	
30– 45	5	1.3	15	0.53	3.6	1.9		120–100	72	
45– 75	6	2.7	30	0.55	3.2	1.8		100– 80	62	
3: 17								25	42	
3: 19	Heart beat stopped. Died.									

Room temperature 8–9°C.
Body temperature 38.4–38.5°C.

Exp. 4. 28. I. 1944 Dog ♂ 13.0 kg.

23.XII. 1943 D_{11}-L_3 dorsal spinal roots sectioned. (16.5 kg.)

10: 40 a.m.—0: 35 p.m. Left lumbar route preparation. Left femoral artery and right saphena vein prepared.

2: 40	p.m.	1	4.1	30	0.63	0.1	0.06	0.074	110	120	12
		2	4.1	30	0.63						
2: 50		3	4.3	30	0.66	0.1	0.07	0.074	108	135	12

3: 00 KCN (2.5 mg. per kg.) was injected intravenously in 30 seconds.

0– 30	4	4.1	30	0.63	0.1	0.06		110–136–125		
30– 60	5	3.8	30	0.59	3.2	1.9		125– 18		
60– 90	6	3.1	30	0.48	0.6	0.29		18– 62	28	
90–120	7	2.6	30	0.40	0.05	0.02		46	32	
120–150	8	3.0	30	0.46	0.05	0.02		46	62	
3: 04								30	66	
3; 05	Heart beat stopped. Died.									

Room temperature 13°C.
Body temperature 37.7°C.

Exp. 5. 14. X. 1943 Dog ♂ 13.8 kg.

18. IX. 1943 D_{11}-L_3 dorsal spinal roots sectioned. (13.8 kg.)

9.50 a.m.—0: 10 p.m. Left lumbar route preparation. Left femoral artery and right

saphena vein prepared.

2: 55 p.m.	1	2.2	30	0.32	0.05	0.02	0.114	93	108	15
	2	2.3	30	0.33						
3: 05	3	2.4	30	0.35	0.05	0.02	0.111	99	111	15
3: 15	KCN (1.5 mg. per kg.) was injected subcutaneously.									
3: 25	4	1.6	30	0.23	0.1	0.02	0.111	79	144	20
	5	1.8	30	0.26						
3: 35	6	1.6	30	0.23	0.1	0.02	0.085	80	132	21
	7	1.7	30	0.25						
3: 45	8	1.6	30	0.23	0.075	0.02	0.085	80	144	20
	9	1.7	30	0.25						
3: 55	10	1.3	30	0.19	0.2	0.04	0.095			
	11	1.3	30	0.19						
4: 00								78	132	18
4: 15	12	1.3	30	0.19	0.05	0.01	0.091	78	156	18
	13	1.2	30	0.17						
4: 45							0.091			

Room temperature 18–20°C.
Body temperature 38.3–39.0°C.

Exp. 6. 5. V. 1944 Dog ♀ 12.8 kg.
7. V. 1944 D_{11}-L_2 dorsal spinal roots sectioned. (14.7 kg.)
9: 50 a.m.—11: 10 a.m. left lumbar route preparation. Left femoral artery and right saphena vein prepared.

2: 00 p.m.	1	3.5	30	0.55	0.05	0.03	0.102	77	162	19
	2	3.0	30	0.47						
2: 10	3	3.8	30	0.59	0.05	0.03	0.099	78	132	18
2: 15	KCN (3.0 mg. per kg.) was injected subcutaneously.									
2: 20								69	198	18
2: 25								82	210	24
2: 30	4	2.9	30	0.45	0.05	0.02	0.108			
2: 35								60	192	33
3: 10	5	3.6	30	0.56	0.05	0.03	0.117	49	162	21
	6	3.9	30	0.61						
3: 15	7	2.5	20	0.61	0.05	0.03				
3: 15	KCN (4.0 mg. per kg.) was injected subcutaneously.									
3: 20	8	2.9	20	0.68	0.05	0.03	0.099	58	180	24
3: 45	9	2.6	30	0.41	0.1	0.04	0.143			
3: 50	10	2.6	30	0.41	0.15	0.06	0.164	77	168	40
3: 52	11	3.1	30	0.49	0.8	0.39	0.168	92	168	54
4: 10	12	2.8	30	0.44	0.7	0.31	0.173	58	156	36

Room temperature 18–19°C.
Body temperature 39.4–40.0°C.

was observed. 5 minutes later the intensive convulsion took place on whole body, which lasted some minutes. The heart rate and the frequency of respiration increased. Concomitantly with the occurrance of convulsion, the blood pressure elevated and it descended again concomitantly with the disappearance of convulsion. The adrenaline secretion rate remained

unaltered till the convulsion set in. It was 0.02–0.04 γ per kg. per minute. The specimen taken during convulsion, the accelerated adrenaline secretion such as 0.39 γ per kg. per minute was observed. In the next specimen, which was taken about 20 minutes later, when the animal was quite quiet, the acceleration of adrenaline secretion was still observed. It was 0.31 γ per kg. per minute. The blood sugar content was 0.099–0.102% before injection, and increased insignificantly after the first injection. After the second injection it commenced to increase earlier than the augmentation of adrenaline secretion. 0.143% was estimated some minutes before the occurrence of convulsion, while the adrenaline secretion was still unaltered. The blood sugar content increased thereafter gradually and at the end of this experiment, namely about one hour after the second injection it was 0.173%.

Summary.

The suprarenal vein blood was sampled in non-anesthetized dogs and the adrenaline in the suprarenal vein blood was estimated by the rabbit intestine segment method. Potassium cyanide was injected in a dose of 1.0–2.5 mg. per kg. intravenously or 1.5–4.0 mg. per kg. subcutaneously.

After injection when the symptoms of cyanide poisoning were manifested, the secretion of adrenaline accelerated decidedly. An enormously accelerated adrenaline secretion such as 1.9 γ per kg. per minute was able to observe in the cases, in which potassium cyanide was injected in a dose of 2.0 mg. or 2.5 mg. per kg. intravenously.

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Is it Adequate to Express the Content of Adrenaline in Adrenals in the Term of Weight, of Gland or of Animal Body?

By

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A comparison can be properly done of the content of adrenaline in the adrenal gland provided it is computed on the weight, not of the gland but of the body. This is a view put forward by H. Sato on the base of a large number of rabbits, such as six hundreds, and in fact on the data obtained only by one and the same method of determination, i. e. by the Suto corrosive sublimate method. 0.56 mg. per g. gland, V 40%, and 0.088 mg. per kg. body, V 24% are the figures.[1]

Is it now then justifiable to generalize this view for other or all kinds of animal species?

In trying to answer this query I have searched for the data presented by previous writers. Contrary to our expection there are only very few data available in the literature. However, it has been largely supplemented by the manuscript of Dr. T. Kojima and others, left in the Laboratory, in which the body weight and the age of animals are noted, besides the figures published at their time.[2]

The figures arrived at here are by no means abundant, but sufficient, I believe, to give a definite conclusion for this problem.

The sex, age, body and gland weight of the animals of Kojima *et al.* are: Cattle (10 individuals, Tab. VI.) No. 1: ♂, 375 kg., 3y., 9.5 g.; No. 2: ♀, 412 kg., 5y., 10.5 g.,; No. 3: ♂, 450 kg., 3y., 15.0 g.; No. 4: ♀, 375 kg., 5y., 9.0 g.; No. 5: ♀, 412 kg., 8y., 21.5 g.; No. 6: ♀, 300 kg., 3y., 8.5 g.; No. 7: ♂, 349 kg., 3y., 8.0 g.; No. 8: ♂, 544 kg., 5y., 9.5 g.; No. 9: ♀, 536 kg., 6y., 22.0 g.; No. 10: ♀, 450 kg., 6y., 18.0 g.; Horse (6 individuals, Tab. IX.) No. 1: ♂, 300 kg., 10y., 14.0 g.; No. 2: ♀, 262.5 kg., 15y., 12.0 g.; No. 3: ♂, 281.3 kg. 17y., 15.0 g.; No. 6: ♂, 300 kg.: 10y., 10 g.; Pig (10 individuals, Tab. IV.) No. 1: ♂, 75.0 kg., 4.0 g.; No. 2: ♂, 67.5 kg., 4.0 g.; No. 3: ♀, 135.0 kg., 6.0 g.; No. 4: ♀, 82.5 kg., 3.5 g.; No. 5: ♀, 67.5 kg., 6.0 g.; No. 6: ♀, 97.5 kg., 6.0 g.; No. 7:

♀, 101.0 kg., 3.0 g.; No. 8: ♂, 56.3 kg., 5.5 g.; No. 9: ♀, 75.0 kg., 4.0, g.; No. 10: ♂, 97.5 kg., 3.0 g.

Colorimetrical methods used: Battelli[3] (dogs, sheep, pigs, horses, cattle), iron chloride; Kuriyama[4] (rats), the Folin; Fujii,[5][6] (rabbits, dogs) the Folin; Kojima, Nemoto, Saito, Sato and Suzuki (cattle, horses, pigs), the Folin and the Suto.

All the data are epitomized in the accompanying table; the mathematical treatment was done under guidance of Dr. Kozo Hirota, to whom my sincere thanks are due.

Table I.

Showing the Adrenaline Content in Terms of Unit of the Gland or That of Body.

Adrenaline content is indicated in mg., the gland weight in g and the body weight in kg The width of variation is expressed in percentage. M: Mathematical mean. σ: Standard deviation. V: Variation coefficient.

		By the Suto method						By the Folin method					
		Adr./Gl.			Adr./B.W.			Adr./Gl.			Adr./B.W.		
		M	σ	V	M	σ	V	M	σ	V	M	σ	V
Kojima *et al.*	Cattle (10)	2.9	0.73	25%	0.086	0.025	30%	3.4	0.73	22%	0.101	0.032	31%
	Horse (6)	2.2	0.34	16%	0.106	0.022	21%	2.8	0.56	20%	0.136	0.030	22%
	Pig (10)	1.8	0.63	35%	0.050	0.016	32%	2.5	0.41	16%	0.15	0.06	42%
Sato-Rabbit (617)		0.56	0.22	40%	0.088	0.021	24%						
Fujii-Rabbit (148 male)								1.13	0.33	29%	0.237	0.087	31%
Fujii-Dog								1.8	0.44	25%	0.22	0.051	24%
Kuriyama-Rat								2.2	0.58	27%	0.043	0.014	32%

		By the iron chloride method					
		Adr./Gl.			Adr./B.W.		
		M	σ	V	M	σ	V
Battelli	Dog (5)	1.03	0.34	33%	0.082	0.020	25%
	Sheep (2)	1.45	0.07	4.8%	0.118	0.004	3.6%
	Pig (2)	1.85	0.07	3.8%	0.081	0.004	5.4%
	Cattle (2)	1.7	0.0	0	0.076	0.003	3.5%
	Horse (4)	1.10	0.24	22%	0.97	0.16	1.7%

A glance at the table shows generally that the adrenaline content varies individually within about thirty percent in all the animal species, and whether it will be experessed in terms of the gland weight or the body weight, has no significant bearing upon the picture.

Nevertheless H. Sato's view can be never too fully appreciated. The rabbit makes an exceptional case; the cortical mass is abundant, twenty

fold of the medullary, and might vary from case to case. Accessory cortical mass exists invariably in this species of animal. The figures obtainable by the Folin method show a rather diagnal tendency (Cf. Tab.); the ascorbic acid in the cortical tissue is thereby assayed together.—About $1^0/_{00}$.[7]

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Studies on the Temperature of the Gastrointestinal Tract.

First Report:

Intra-gastric and -esophageal Temperatures of Healthy Adults.

By

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(Received for publication, May 23, 1951)

H. Necheles[1] in the section on "Intragastric Temperature" in S. A. Portis's monograph, "Diseases of the Digestive System," quoted and discussed the results of J. S. Hepburn and his co-workers. He advocated that changes of intragastric temperature to presume vasomotor reactions or blood flows in the stomach, are related to the etiology of the peptic ulcer. Although researches on the intragastric temperature have increased, studies have been directed hitherto to other physiological problems of the stomach. Previous studies on the said temperature were almost limited to measurements of its normal range,[2)-7)] observations on its changes by ingestions of foods and drinks,[2)3)4)5)7)] local applications of heat and cold on the epigastrium;[2)3)5)8)] systematical and comparative studies were few. Our knowledges on the temperatures of pathological stomachs and the alimentary tract except the stomach, especially the esophagus and small intestine, are scanty. In concern with the above mentioned, the necessity for systematical studies on the temperature of the gastrointestinal tract is definite.

Since April 1949, I have been studying the temperature of the gastrointestinal tract of the human body, and as the first report, I shall herein describe the method of measurement of the intra-gastric and -esophageal temperatures. The data and some discussions as presented concern healthy adults.

Experimental.

Methods.

Of the thermoelectric and the resistance thermometers used to measure the inner temperatures of the human body, the latter was employed after

various tests, even though both have mutually strong as well as weak points.

The resistance thermometer constructed by us consists of a thermometer which can be swallowed as a stomach tube (Fig. 1), and an indicator (Fig. 2). In Fig. 1, A is the resistance bulb, 18 mm in length and 7.5 mm

Fig. 1. Thermometer.

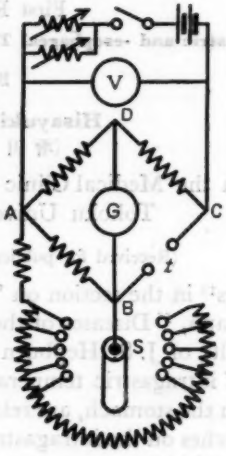

Fig. 2. Indicator.

in outside diameter. It is an amber cylinder on which a fine platinum wire, 0.02 mm in diameter and 70 ohms in electrical resistance, is coiled, and on the top a hemispherical brass B is attached to make it visible at fluoroscopy. C is the Miller-Abbott's tube, 100 cm in length; one tube leads from A to the terminal T, and through the other, the stomach contents are drawn out, or water and solution are introduced into the stomach from the opening E. In Fig. 2, the quadrilateral ABCD is the Wheatstone's bridge, to which 2 volts are loaded after attachment of T to the terminal t of the side BC. One of the parallel resistances of the measuring arm AB is made gradational by switch S, the differences of which are further read by an unbalance current of the bridge, i. e., galvanometer G (10^{-8} A). The whole are mounted in a box measuring $38 \times 33 \times 30$ cm for carrying. This thermometer has a temperature range from O to 50° C and 3 readings of the galvanometer scale correspond to 0.1° C.

Before use of this thermometer, calibration must first be accomplished with the standard mercury thermometer, both of the number of the switch S and the reading of the galvanometer scale are noted and then the temperature is determined.

Results.

From September 1949 to November 1950 I measured 180 healthy adults in the fasting state, at least 4 hours after meals. The intragastric temperature was taken when the resistance bulb was swallowed and measured 60 cm from the incisor of the upper jaw, and the intraesophageal was 30 cm. Simultaneously the axillary temperature was measured by the mercury thermometer.

The maximums, minimums, means and 0.95 fiducial limits of the means of the intra-gastric, -esophageal and axillary temperatures of 180 cases which included 148 men and 32 women are shown in Table I.

TABLE I.

Intra-gastric, -esophageal and Axillary Tempertures of 180 Healthy Adults (148 men and 32 women).

	Item	Sex	Number cases	Maximum °C	Minimum °C	Mean °C	0.95 fiducial limits of mean °C
1	Intra-gastric temp.	♂+♀	180	38.0	36.3	37.29	37.29±0.05
2		♂	148	38.0	36.3	37.24	37.24±0.05
3		♀	32	37.9	36.6	37.54	37.54±0.10
4	Intra-esophageal temp.	♂+♀	180	37.8	36.0	36.77	36.77±0.06
5		♂	148	37.8	36.0	36.68	36.68±0.06
6		♀	32	37.6	36.3	37.16	37.16±0.10
7	Axillary temp.	♂+♀	180	37.1	35.3	36.35	36.35±0.06
8		♂	148	37.1	35.3	36.34	36.34±0.06
9		♀	32	37.1	35.3	36.39	36.39±0.14

The results of measurement of the above metntioned men and women, before breakfast, lunch and dinner, are given in Table II.

The room temperature under which measurements were performed, was in the range of 10–16° C, 17–20° C and 21–26° C, as shown in Table III.

The results of measurement carried out on three same men, 20 times during 11 months at the same time daily i. e., before lunch, are given in Table IV.

The intra-gastric and -esophageal temperatures measured as above were almost the same as those measured by previous workers.

Discussion.

When the temperature distributions of more than 30 or 40 individuals given in Tables I, II and III, are plotted on the probability paper they become almost linear. It is certain that the distributions of the intra-gastric, -esophageal and axillary temperatures show respectively normal distri-

Table II.

Intra-gastric, -esophageal and Axillary Temperatures before Breakfast, Lunch and Dinner.

	Item	Time	Sex	Number cases	Maximum °C	Minimum °C	Mean °C	0.95 fiducial limits of mean °C
10	Intra-gastric temp.	Before breakfast	♂+♀	42	37.6	36.4	37.12	37.12±0.11
11			♂	35	37.6	36.4	37.07	37.07±0.12
12			♀	7	37.6	36.6	37.36	37.36±0.32
13		Before lunch	♂+♀	104	37.9	36.3	37.29	37.29±0.05
14			♂	94	37.9	36.3	37.27	37.27±0.05
15			♀	10	37.9	37.1	37.46	37.46±0.18
16		Before dinner	♂+♀	34	38.0	36.7	37.54	37.54±0.10
17			♂	19	38.0	36.7	37.43	37.43±0.16
18			♀	15	37.9	37.4	37.67	37.67±0.13
19	Intra-esophageal temp.	Before breakfast	♂+♀	42	37.4	36.0	36.62	36.62±0.12
20			♂	35	37.2	36.0	36.53	36.53±0.11
21			♀	7	37.4	36.3	37.07	37.07±0.36
22		Before lunch	♂+♀	104	37.4	36.0	36.71	36.71±0.06
23			♂	94	37.4	36.0	36.68	36.68±0.06
24			♀	10	37.4	36.5	36.93	36.93±0.17
25		Before dinner	♂+♀	34	37.8	36.0	37.14	37.14±0.14
26			♂	19	37.8	36.0	36.98	36.98±0.22
27			♀	15	37.6	37.1	37.34	37.34±0.11
28	Axillary temp.	Before breakfast	♂+♀	42	37.0	35.3	36.07	36.07±0.14
29			♂	35	37.0	35.3	36.08	36.08±0.16
30			♀	7	36.7	35.3	36.00	36.00±0.40
31		Before lunch	♂+♀	104	37.1	35.4	36.39	36.39±0.06
32			♂	94	37.1	35.4	36.39	36.39±0.07
33			♀	10	36.9	36.0	36.39	36.39±0.21
34		Before dinner	♂+♀	34	37.1	35.9	36.58	36.58±0.11
35			♂	19	37.1	35.9	36.59	36.59±0.15
36			♀	15	37.1	36.0	36.57	36.57±0.18

Table III.

Intra-gastric, -esophageal and Axillary Temperatures in the Three Groups according to the Room Temperature.

	Item	Room temp. °C	Number cases	Maximum °C	Minimum °C	Mean °C	0.95 fiducial limits of mean °C
37	Intra-gastric temp.	10–16	61	38.0	36.3	37.22	37.22±0.10
38		17–20	66	37.9	36.9	37.37	37.37±0.07
39		21–26	53	37.9	36.6	37.28	37.28±0.08
40	Intra-esophageal temp.	10–16	61	37.8	36.0	36.79	36.79±0.07
41		17–20	66	37.6	36.1	36.83	36.83±0.09
42		21–26	53	37.5	36.0	36.76	36.76±0.09
43	Axillary temp.	10–16	61	37.0	35.3	36.19	36.19±0.12
44		17–20	66	37.1	35.4	36.42	36.42±0.09
45		21–26	53	37.1	35.7	36.46	36.46±0.09

TABLE IV.

Intra-gastric, -esophageal and Axillary Temperatures of the Three Same Men Measured 20 Times in 11 Months at the Same Time Daily.

	Item	Name	Maximum °C	Minimum °C	Mean °C	0.95 fiducial limits of mean °C
1	Intra-gastric temp.	H.M.	37.7	36.8	37.20	37.20±0.12
2		M.O.	37.7	36.9	37.22	37.22±0.13
3		S.K.	37.6	36.6	37.14	37.14±0.14
4	Intra-esophageal temp.	H.M.	37.3	36.1	36.67	36.67±0.13
5		M.O.	37.0	36.4	36.61	36.61±0.09
6		S.K.	37.1	36.2	36.52	36.52±0.12
7	Axillary temp.	H.M.	36.9	36.0	36.52	36.52±0.12
8		M.O.	36.6	35.6	36.33	36.33±0.12
9		S.K.	37.0	36.0	36.32	36.32±0.11

H. M.: ♂ 29 years old.
M. O.: ♂ 28 years old.
S. K.: ♂ 27 years old.

butions in the cases when they were divided in before breakfast, lunch and dinner, men and women, room temperature, and when observed undivided as above. This means that the nearer the three temperatures are to their mean, the larger their frequency becomes. As a normality of the temperature distributions was recognized, the following discussions are brought forth.

It was tested whether the differences among the intra-gastric, -esophageal and axillary temperatures are statistically significant or not. In Tables I, II and III, there are 15 combinations of the corresponding three temperatures, i. e., 1, 4 and 7; 2, 5 and 8; ...; 37, 40 and 43; ... etc. In each of them statistically significant differences among the means of the three temperatures were confirmed. The intragastric temperature was 0.2–0.6° C higher than the intraesophageal and the latter was 0.3 –1.0° C higher than the axillary.

As the differences of the three temperatures statiscally were recognized, then the correlations among them were analyzed. In 180 individuals, the coefficient of correlation between the intra-gastric and -esophageal, r_{xy}, that between the intraesophageal and axillary, r_{yz}, and that between the axillary and intragastric, r_{zx}, were as following:

$r_{xy}=0.8146$, $r_{yz}=0.4438$, and $r_{zx}=0.5248$.

These coefficients of correlation were all statistically significant, and r_{xy} significantly exceeded r_{yz} and r_{zx}, but the difference between r_{yz} and r_{zx} was not significant. In short, among the three temperatures there were mutually positive correlations; a strong one between the intra-gastric and -esophageal, and moderate ones between the axillary and the remaining two.

As the difference in the body temperature between men and women has been discussed, from the obtained data it was tested. In the four cases of the intragastric temperature; men and women, i. e., 2 and 3 in Table I, and those divided into before breakfast, lunch and dinner, i. e., 11 and 12, 14 and 15, and 17 and 18, in Table II; it is confirmed that there are statistically significant differences between men and women, being higher in women than men by 0.2–0.3° C. In the intraesophageal temperature the differences were the same as the intragastric except by 0.3–0.4° C, but in the axillary temperature the differences were not significant in all four cases.

It has been said that there is a diurnal variance in the body temperature. As measurings were carried out before breakfast, lunch and dinner, the diurnal variance was analyzed by comparing these three groups. The intragastric temperatures are divided into men and women, i. e., 11, 14 and 17, and 12, 15 and 18 in Table II, or not, i. e., 10, 13 and 16, among the three groups statistically significant differences are mutually confirmed. The intraesophageal and axillary temperatures were the same as the intragastirc. It was 0.1–0.4° C higher before lunch than breakfast, and 0.2–0.4° C higher before dinner than lunch.

Then the temperature gradients of the three temperatures were analyzed, when they were divided into before breakfast, lunch and dinner. In Fig. 3 three broken lines are obtained by plotting each set of three points,

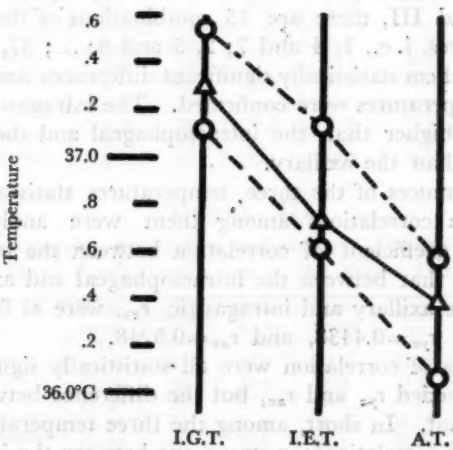

Fig. 3. Temperature gradients of intra-gastric (I.G.T.), -esophageal (I.E.T.) and axillary temperatures (A. T.), before breakfast (– – – –), lunch (————) and dinner (– – – – – – –).

i.e., 10, 19 and 28; 13, 22 and 31; and 16, 25 and 34 in Table II; statistically significant differences among these three broken lines were confirmed. The temperature gradients of the three temperatures also vary in the course of a day.

Whether the three temperatures differed by the room temperature under which measurings were carried out, was tested. Among the intragastric temperature of the three groups; 10–16° C (37 in Table III), 17–20° C (38) and 21–26° C (39), statistically significant differences were not seen. It was the same in the intraesophageal temperature as in the intragastric. In the axillary temperature the group of 21–26° C was significantly higher than the two others by 0.2–0.3° C.

Further the correlation between the room temperature and the three temperatures was analyzed, and their coefficients r_{xt}, r_{yt} and r_{zt} were as follows:

$r_{xt}=0.1084$, $r_{yt}=-0.0366$, and $r_{zt}=0.2858$.

Among the three coefficients only r_{zt} was statistically significant. r_{zt} was significantly greater than r_{xt} and r_{yt}, but the difference between r_{xt} and r_{yt} was not significant. In short, the intra-gastric and -esophageal temperatures had no correlation with the room temperature, but the axillary showed weak positive correlation therewith.

The results of measurement of the three same men performed 20 times during 11 months at the same time daily are shown in Table IV, from which it is clear that the three temperatures are each variable by about 1° C from one day to another. Among the three men the intra-gastric and -esophageal temperatures showed no statistically significant difference, but the axillary showed significant difference between the two (H. M. and S. K.) of the three. It is presumable that the individual variation is most easily observed in the axillary temperature.

Summary.

I have described a precise method to measure the intra-gastric and -esophageal temperatures, and by this thermometer the intra-gastric and -esophageal temperatures on 180 healthy adults were simultaneously measured with the axillary. From the obtained results the following facts were confirmed.

1. The intra-gastric, -esophageal and axillary temperatures exhibit normal distribution.

2. The three temperatures have mutually distinct differences. The intragastric was 0.2–0.6° C higher than the intraesophageal and the latter was 0.3–1.0° C higher than the axillary.

3. Among the three temperatures there are mutually positive correlations; a strong one between the intra-gastric and -esophageal, and

moderate ones between the axillary and the remaining two.

4. The intra-gastric and -esophageal temperatures were higher in women than men by 0.2–0.4° C, but in the axillary the difference between them was not significant.

5. In the three temperatures there are diurnal variances; being 0.1–0.4° C higher before lunch than breakfast, and 0.2–0.4° C higher before dinner than lunch. The temperature gradient of the three temperatures also vary in the course of a day.

6. The intra-gastric and -esophageal temperatures were independent on the room temperature but the axillary was 0.2–0.3° C higher in the room temperature of 21–26° C than in one of 10–20° C.

7. The intra-gastric and -esophageal temperatures have no correlation with the room temperature, but the axillary showed a weak positive correlation with it.

8. In the same man the three temperatures each vary by about 1°C from one day to another and the individual variation is most easily observed in the axillary.

For the construction of the apparatus used in the present study, the author expresses his deep appreciation to the members of the Research Institute for Sciencific Measurements of Tohoku University.

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Studies on the Temperature of the Gastrointestinal Tract.

Second Report:

Influence of Bathing on the Intragastric Temperature.

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Researches on the changes of the intragastric temperature of the human body by taking a bath are scanty, for which reason we have carried out the present study at the Narugo Spa in Miyagi Prefecture in November 1949.

Experimental.

Methods and Materials.

We made experiments at the following four hot-springs of the Narugo Spa: (1) the earthy sulphate spring (pH 6.6), (2) the alkaline muriated saline sulphur spring (pH 9.2), (3) the sulphuretted acid vitriol spring (pH 1.2), and (4) the sulphuretted simple carbondioxated spring (pH 5.8). A hot bath of tap-water was also used as a control. On the 18 healthy adults (14 men and 4 women) 46 experiments were performed. Continuously before, during and after the bath, we measured the intragastric temperature at 60 cm from the incisor of the upper jaw, in the fasting state at least 4 hours after meals. After bathing, it was measured in the bath room at about 15° C without putting on clothes for more than 30 minutes. In the bath the whole body except the head was submerged. For measurements of the intragastric temperature the thermometer described in the previous paper[1] was used and for the axillary the mercury thermometer.

If among the three factors of the bath, i. e., the kind of spring, temperature and time of the bath, two or one are made constant and the remaining one or two variable, then several combinations of the three are given. By making experiments according to each of the combinations, influences of bathing on the intragastric temperature were studied. Some other experiments were also made. The object of this study was chiefly the hot bath above 40° C.

Results and Discussion.

1) The combination of the constant temperature and kind of spring, and variable times.

The rises of the intragastric temperatures of 10 individuals who bathed in the sulphate spring for 8 minutes at 44° C are given in Table I and Fig.

Table I.

The Rises of the Intragastric Temperature in the Sulphate Spring at 44°C for 8 Minutes.

No.	Before the bath	During the bath (minutes)							
		1	2	3	4	5	6	7	8
	°C	°C	°C	°C	°C	°C	°C	°C	°C
1	37.1	0	0.1	0.2	0.4	0.5	0.6	0.9	1.1
2	37.4	0	0.1	0.2	0.5	0.7	1.1	1.3	1.7
3	37.8	0	0	0.2	0.4	0.6	0.8	1.2	1.4
4	37.4	0	0.1	0.2	0.4	0.8	0.9	1.1	1.3
5	37.1	0	0	0.2	0.6	0.8	1.1	1.3	1.4
6	37.5	0	0.1	0.3	0.5	0.6	0.6	1.1	1.3
7	37.0	0	0	0	0.1	0.2	0.5	0.6	0.9
8	37.0	0.1	0.2	0.3	0.5	0.7	0.7	0.9	1.1
9	37.6	0	0	0.1	0.2	0.5	0.7	0.9	0.9
10	37.6	0	0	0.2	0.4	0.7	1.0	1.3	1.6
Sum	373.5	0.1	0.6	1.9	4.0	6.1	8.2	10.6	12.7
Mean	37.35	0.01	0.06	0.19	0.40	0.61	0.82	1.06	1.27

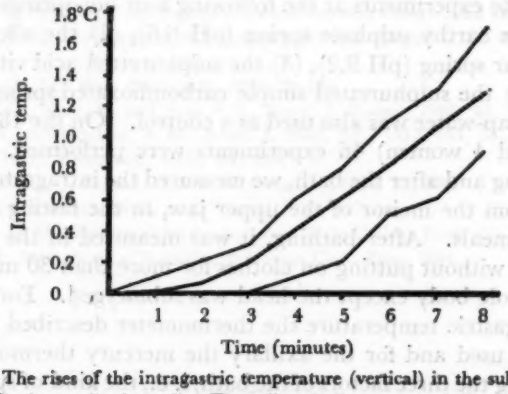

Fig. 1. The rises of the intragastric temperature (vertical) in the sulphate spring at 44°C for 8 minutes (horizontal). Three lines indicate respectively the maximum, minimum and mean of the rises.

1. In Fig. 1 the maximums, minimums and means of the rises of every minutes are plotted. In these 10 cases the rise of the intragastric temperature observed from the values before, and 1 and 2 minutes during the bath, was not statistically significant, contrary to the extended time of

2 and 3 minutes which was significant. The rise becomes first significant in the course of more than 2 minutes. As the type of the rise is almost linear obviously in Fig. 1, the relation between the rise and the time during the bath is represented by a straight line. If the rise in t minutes during the bath is X° C, X is given according to the empirical formula as follow:

$$X=0.22\,t-0.47\ (t>2).$$

2) The combination of the constant kind of spring and time, and variable temperatures.

The rises of the intragastric temperatures of the three same men who bathed 4 minutes in the sulphate spring at 40, 44 and 47° C, and of the five same men at 43 and 44° C, are shown in Table II and Fig. 2. The

TABLE II.

The Rises of the Intragastric Temperature in the Sulphate Spring for 4 Minutes at 40, 43, 44 and 47° C.

No.	A 40°C	B 44°C	C 47°C	D 43°C	E 44°C
	°C	°C	°C	°C	°C
1	0.1	0.4	0.9	0.1	0.4
2	0	0.5	0.5	0.2	0.5
6	0	0.5	1.5	0.4	0.5
11				0.1	0.5
12				0.1	0.4
Sum	0.1	1.4	2.9	0.9	2.3
Mean	0.03	0.47	0.97	0.18	0.46

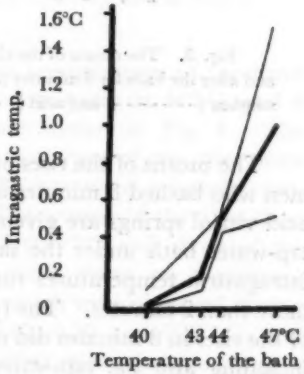

Fig. 2. The rises of the intragastric temperature (vertical) in the sulphate spring for 4 minutes at 40, 43, 44 and 47°C (horizontal). Three lines indicate respectively the maximum, minimum and mean of the rises.

rise in 4 minutes at 40° C was not statistically significant, but those at 43° C and above were all significant. Between the means of the rises at 40° C (A in Table II) and 44° C (B), those at 44° C (B) and 47° C (C), and those at 43° C (D) and 44° C (E), significant differences were seen. It is certain that the rise of the intragastric temperature is directly proportional to the temperature of the bath, and 43° C is the lower level of the rise in a bath for 4 minutes.

3) The combination of the constant temperature and time, and different kinds of spring.

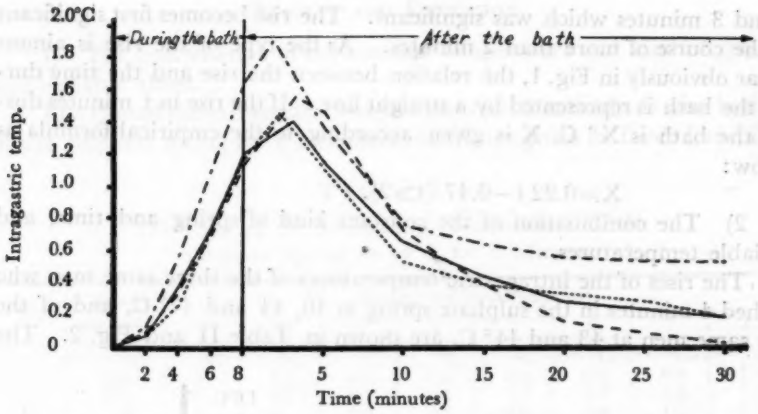

Fig. 3. The means of the changes of the intragastric temperature (vertical) during and after the bath for 8 minutes (horizontal) at 44°C in the sulphate (————), alkaline sulphur (– – – – –) and acid (· · · · · ·) springs and in the tap-water bath (– – – – –).

The means of the rises of the intragastric tmeperatures of the six same men who bathed 8 minutes at 44° C in the sulphate, alkaline sulphur and acid vitriol springs are given in Fig. 3. The means of 4 individuals of the tap-water bath under the same conditions are shown as a control. The intragastric temperatures rose linear as above mentioned in the course of more than 2 minutes. The types of the rises during the bath and the means of the rises in 8 minutes did not differ significantly by the 4 kinds of spring including also the tap-water bath. The intragastric temperatures continued to rise during the first several minutes after bathing and reached the maximum rises. The mean of the maximum rise in the alkaline sulphur spring was 1.90°C, thus being significantly higher than those in the sulphate (1.40° C) and acid springs (1.45° C). That in the tap-water bath was 1.55° C. The fall of the intragastric temperature thereafter in the alkaline sulphur spring, observed from the values before bathing, of the maximum rise and 30 minutes after bathing, was significantly slower than those at the other three. The mean of the intragastric temperature 30 minutes after bathing in the alkaline sulphur spring was still 0.52° C higher than that before bathing. This difference was significantly greater than those in the sulphate (0.18° C), acid springs (0.18° C) and tap-water bath (0.20° C). These are interesting compared with the fact that at the Narugo Spa, warmth after bathing is said to remain longest in this alkaline sulphur spring.

4) The combinatio n of the constant kind of spring, and variable temperatures and times.

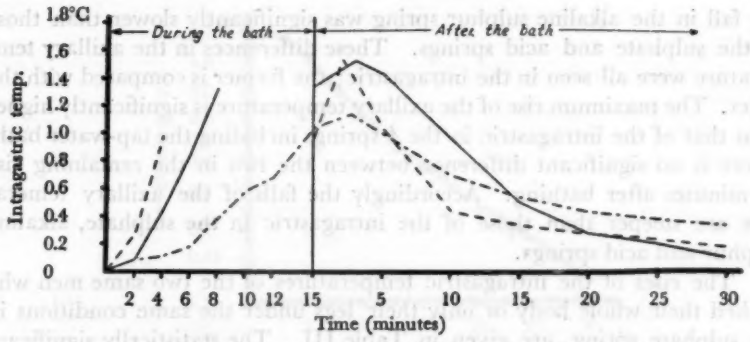

Fig. 4. The means of the changes of the intragastric temperature (vertical) during and after the bath (horizontal) for 15 minutes at 40°C (--------), for 8 at 44°C (-- ---) and for 4 at 47°C (———).

The means of the rises of the intragastric temperatures of the three same men who bathed 15 minutes at 40° C, 8 minutes at 44° C, and 4 minutes at 47° C in the sulphate spring are shown in Fig. 4. The rises were linear as above mentioned. But the types of the rises were obviously different and their direction coefficients were generally proportional to the temperature of the bath. The means of the rises soon after bathing were respectively 1.03, 1.33 and 0.97° C and those of the maximum rises 1.13, 1.53 and 1.53° C. Among them no significant differences were seen. The means of the rises which were observed during the first several minutes after bathing were 0.10, 0.20 and 0.57° C, and the latter was significantly greater than the other two. In this group a stimulation by bathing is so strong that the after-effect is intense. The fall of the intragastric temperature after bathing did not differ by the three groups in these three men. In the group of 15 minutes at 40° C, as obviously seen in Fig. 4, the fall seems to be slowoer and the intragastric temperature 30 minutes after bathing still remains higher than the other two. This seems to be related to the fact that warmth after bathing remains longer by taking a long bath in a lower temperature.

As in the case of the above cited 3), the axillary temperatures after bathing were measured, the results are briefly described and compared with those of the intragastric. The means of the maximum rise of the axillary temperature in the alkaline sulphur spring (3.10° C) was significantly higher than those in the acid spring (2.40° C) and in the tap-water bath (2.50° C). That in the sulphate spring was 2.78° C. The mean of the rise still remained 30 minutes after bathing in the alkaline sulphur spring was 0.43° C; this was significantly higher than those in the sulphate (0° C) and acid springs (0.08° C) and in the tap-water bath (0° C). Accordingly

the fall in the alkaline sulphur spring was significantly slower than those in the sulphate and acid springs. These differences in the axillary temperature were all seen in the intragastric; the former is compared with the latter. The maximum rise of the axillary temperature is significantly higher than that of the intragastric in the 4 springs including the tap-water bath. There is no significant difference between the two in the remaining rise 30 minutes after bathing. Accordingly the falls of the axillary temerature are steeper than those of the intragastric in the sulphate, alkaline sulphur and acid springs.

The rises of the intragastric temperatures of the two same men who bathed their whole body or only their legs under the same conditions in the sulphate spring, are given in Table III. The statisticrlly significant

TABLE III.

The Rises of the Intragastric Temperature of the Whole Body and the Partial Bath of the Legs in the Sulphate Spring.

No.		The whole body bath	The partial bath of the legs
	The Bath of	°C	°C
1	8 minutes at 44°C	1.9	0.4
2	15 minutes at 43°C	1.8	1.0
Sum		3.7	1.4
Mean		1.85	0.70

difference between the rise by the two methods of the bath was not seen in these two men. However, if more men are measured, it is certain that the rise by the whole body bath will be significantly higher than that by the partial bath of only thier legs. Concerning this problem it is necessary to study the influence of local warming or cooling of various parts of the body upon the intragastric temperature.

We analyzed the type for the fall of the intragastric temperature after bathing, as an example, in the 10 cases in the sulphate spring. In the semi-logarithmic table, Fig. 5, the mean of the remaining rise of the intragastric temperature is given in logarithmic scale as ordinate and the time after bathing as abscissa. Obviously in Fig. 5 two straight lines are obtained before and after the coordinate of 15 minutes. Accordingly the fall of the intragastric temperature is expressed by the two exponential functions except the first several minutes after bathing. The empirical formulas are as follows:

$X=1.84\ e^{-0.109t}$ $(3\leqq t\leqq 14)$, and $X=1.12\ e^{-0.071t}$ $(15\leqq t\leqq 30)$,

in which X°C is the difference of the intragastric temperature between before and t minutes after bathing. In the other springs the empirical

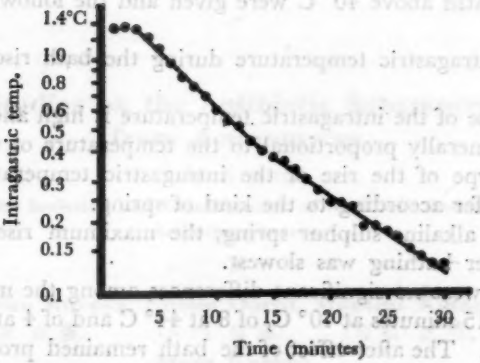

Fig. 5. The means of the falls of the intragastric temperature after bathing in the sulphate spring for 8 minutes at 44°C. The intragastric temperature still remained above the one before bathing (vertical) within 30 minutes after bathing (horizontal). The two lines represent the empirical formulas.

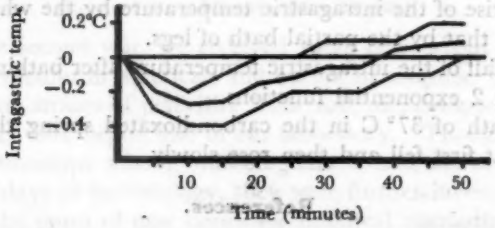

Fig. 6. The changes of the intragastric temperature (vertical) in the carbondioxated spring at 37°C for 50 minutes (horizontal). Three lines indicate respectively the maximum, minimum and mean of the changes.

formulas are represented by more than 2 exponential functions, according to the temperature of the bath and the room temperature after bathing.

The means of the intragastric temperatures of 4 men who bathed 50 minutes at 37° C in the carbondioxated spring, are shown in Fig. 6. The intragastric temperature at first fell and reached the minimum of 0.28° C after 10 minutes. Thereafter it slowly rose and reached after 40 minutes the level before bathing and after 50 minutes it became 0.10° C higher. Both the fall and rise are significant and both augment linear with the time of the bath.

Summary.

The changes of the intragastric temperature of the human body by taking a bath were studied at the four hot-springs of the Narugo Spa. The

results of the baths above 40° C were given and the following facts were confirmed.

1. The intragastric temperature during the bath rises linear with the time.

2. The rise of the intragastric temperature is high and its direction coefficient is generally proportional to the temperature of the bath.

3. The type of the rise of the intragastric temperature does not significantly differ according to the kind of spring.

4. In the alkaline sulphur spring, the maximum rise was highest and the fall after bathing was slowest.

5. There were no significant differences among the maximum rises of the baths of 15 minutes at 40° C, of 8 at 44° C and of 4 at 47° C, in the sulphate spring. The after-effect of the bath remained proportionally to the temperature of the bath.

6. The changes of the axillary temperature have the same tendency as those of the intragastric. The maximum rise was about 1.0° C higher and the fall was steeper in the former than in the latter.

7. The rise of the intragastric temperature by the whole body bath is higher than that by the partial bath of legs.

8. The fall of the intragastric temperature after bathing is expressed by more than 2 exponential functions.

In the bath of 37° C in the carbondioxated spring the intragastric temperature at first fell and then rose slowly.

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Studies on the Antibiotic Substances from Actinomyces.

1st Report.

On the Isolation and Classification of Antibiotic Producing Actinomycetaceae.

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During the second war, we received some news about the practical usefulness of penicillin in american and european countries and to isolate such antagonistic strains of penicillia from soil, air, compost et al., some systematic works were begun also in our laboratory. Though the antagonistic interrelationships among microorganisms had attracted attention since the early days of bacteriology, they were further investigated during the war from the point of new desire for practical chemotherapeutic application.

From fungi, from actinomyces, and from spore bearing, gram positive bacilli many noticeable antibiotics were isolated in this laboratory, viz. from fungi penicillin (Kondo)[1], patulin (Hamagami),[2] fumigacin-like new substance (Kondo and Takahashi),[3] fumigatin-like new substance,[4] spinulosin (Hosisima),[4] and penicillin-like substance from Asperigllus candidus (Ishida)[5] etc.

Also from spore forming bacilli, by Okabe, butanol soluble new non-toxic substance (bacitracin-like)[6] and alcohol soluble polypeptide-like substance[7] (Yamane) were isolated.

From actinomyces we endeavoured to find also some antibiotic agents such as actinomycin-like substance (Akutsu),[8] streptothricin-like substance (Itakura)[9] and streptomycin-like substance (Seki).[10]

All of the above works were performed until 1946, but at that time there was no proper opportunity for the publications owing to unfavourable financial circumstances of this country after the war.

Since 1947, our studies on the antibiotics were chiefly intended to

those produced by actinomyces and a systematic research was begun newly.

The chief results of these works were already reported in Japanese, in "Japanese Journal of Antibiotics (former "Japanese Journal of Penicillin")," but almost all results of our systematic works will be again briefly reported successively in a series in this journal too.

At first in present paper the isolation method and the distribution of actinomyces, and several ways to find antibiotic strains and their classifications will be discussed.

Experimental.

The Isolation Method of Actinomyces from Soil.

It is already generally known[11] that the actinomyces demand about the same amount of C sources as well as bacteria and fungi, but not so much N sources for nutrition. In such a meaning usual isolation media for actinomyces contain generally a rather small amount of N sources. The representative isolation media of actinomyces are as follows:

1) Asparagine glucose agar (Krainsky)[12]

Glucose	10.0 g
Asparagine	0.5 g
K_2HPO_4	0.5 g
Agar	20.0 g
Aq	1000.0 cc

2) Casein glucose agar (Jensen)[12], [13]

Glucose	20.0 g
Casein (dissolved in N/10 NaOH 10 cc)	0.2 g
K_2HPO_4	0.5 g
$MgSO_4 \cdot 7H_2O$	0.2 g
$FeCl_3$	trace
Agar	20.0 g
Aq	1000.0 cc

3) Ammonium glycerol agar (Masumoto)[14]

Glycerol	100.0 g
Ca citrate	10.0 g
NH_4Cl	0.5 g
K_2HPO_4	05. g
Agar	20.0 g
Aq	1000.0 cc

Besides three media mentioned above, albumine agar (Brown), asparagine glycerol agar (Conn), soil agar (Fischer), Czapeck's agar, Ca citrate agar, Ca malate agar, and sodium nitrate agar were used by many authors for isolation. In our experiments, however, the former 3 were used chiefly and their characteristics were compared with each other. All 20 samples of soils were cultivated in a same condition in these media, and the colonies of actinomyces and other microorganisms produced were countered and

shown in Table I. As shown in the table, the colony number of actinomyces grown in casein glucose agar was less than those grown on the other media, whereas the number of the contaminating bacterial or fungal colonies was most in ammonium glycerol agar. From the view of these properties the asparagine glucose agar was found to be more preferable

TABLE I.

The Comparison of 3 Isolation Media.

No. of soil samples	Soils located at	Soil pH	Kind of media		
			Casein glucose agar	Asparagine glucose agar	Ammonium glycerol agar
1	Field	6.0	1*	5	14
2	Field	6.2	21	23	28
3	Hill	6.0	5	7	12
4	Hill	5.8	4	5	10
5	Hill	5.8	2	27	18
6	Field	6.0	1	8	3
7	Paddy field	5.6	0	1	1
8	Hill	5.8	3	7	8
9	Hill	5.8	4	10	6
10	Hill	5.8	2	6	4
11	Hill	6.0	7	14	39
12	Hill	6.0	9	9	0
13	Field	6.2	1	14	14
14	Hill	5.8	24	51	44
15	Field	6.2	1	6	3
16	Hill	5.4	5	13	11
17	Hill	5.8	3	22	19
18	Field	6.6	2	4	5
19	Field	5.8	1	9	12
20	Paddy field	5.4	2	6	10
Total		118	87	247	261
Average		5.9	4.9	12.3	13.0

* 1 g of all soil samples was suspended in 5 cc sterilized physiological saline and shaken well. One platinum loopful of this suspenison was mixed to 10 cc of melted culture media and solidified in Petri dishes. After the incubation for 7 days at 25° the number of the colonies grown in agar media were compared with each other. The numbers indicate those of actinomyces colonies per platinum loop of such a suspension grown in each of 3 media.

than the others. Therefore it was decided to use the asparagine glucose agar for the isolation, preservation and sporulation of actinomyces. According to our experience for 4 years, however, it was thought to be necessary to use also other isolation media, because many antibiotic strains were isolated already by this medium and it seems necessary to use more effective and suitable media in order to isolate new antibiotic strains.

The Distribution of Actinomyces Depending upon the Kinds of Soils.

Fousek,[15] Conn,[16] Waksman,[17],[18] Beierincke[11] and many other authors mentioned already the difference of the number and the rate of actinomyces according to the several kinds of soils and to their depths from where they were taken. In 1947 we collected 188 soil samples from throughout all the territories of Japan and isolated 1233 strains. They were cultivated and preserved on Krainsky's agar slant and almost all of them seemed to belong to Family Streptomycetacesae (Waksman). These 188 soil samples were divided into 3 groups according to the site of sampling, as shown in Table II and it was revealed that larger number of actinomycetes were obtained from field and hill soils, than the others.

The Fluctuation of the Actinomyces According to the Seasons.

Hiltner *et al.*,[19] Fousek,[15] and Waksman reported that more actinomyces were isolated especially in autumn than in other seasons. This fact has been previously recognized by us too and now proved by calculation of the colonies produced by cultivating soil samples taken from selected 20 sites, one sample in each of 4 different seasons respectively, as shown in Table III. Furthermore, 1223 strains isolated from 188 samples, mentioned above were grouped according to the sampling seasons, as shown in Table IV, in order to indicate which season is the best to get more actinomyces colonies. The numbers of colonies obtained were arranged in the order of autumn, spring, summer and winter.

The Number of the Actinomyces Colonies in Relation to the pH of Soils.

The fact that only few colonies of actinomyces will be obtained from acidic soils has been recognized previously. The pH of the soil samples collected from 20 places mentioned above was estimated and shown in Table V, and from the point of view of the averaged pH of each of 3 groups of soil samples being divided according to the location of soil sampling it is indicated that the fewer colonies will be obtained, the more acidic was the pH of soils (Table VI). Moreover each 10 samples of soils of 3 different pH, viz. pH 5.8, 5.6, 5.4 were collected and the numbers of colonies were counted in a similar way, as shown in Table VII. These data proved also that in acidic pH, the number of actinomyces is relatively few.

Jensen[13] reported that, if 1–4 per cent $CaCO_3$ were added to acidic soil samples according to its acidity and preserved in a suitable temperature and in a well aerated condition, a remarkable increase of the actinomyces

TABLE II.

The Distribution of Actinomyces Depending upon the Soil Kinds.

Soils located at	Number of samples	Total number of actinomyces	Averaged number per loop of soil suspension
Field	76	532	6.9
Hills	94	623	6.6
Others	18	68	3.8
Total	188	1223	

TABLE III.

The Distribution of Actinomyces with regards to the Seasons.

Seasons	Total number of actinomyces colonies	Averaged colony number obtained in each one place
Spring	156	7.8
Summer	105	5.2
Autumn	207	10.3
Winter	43	2.1

20 sites were selected for the sampling of soils and one sample was taken from each of these 20 sites in spring, summer, autumn and winter respectively. The cultivation was made in the same manner mentioned above.

TABLE IV.

The Distribution of Actinomyces with regard to the Seasons

Seasons	Number of soil samples	Total colony numbers obtained	Averaged colony number obtained from each one place
Spring	47	365	7.6
Summer	22	103	4.7
Autumn	69	587	8.5
Winter	50	168	3.3
	188	1223	6.02

1223 actinomyces strains obtained from 188 soil samples, as shown in Table II, were divided into 4 groups according to the season, at which they were sampled in order to indicate, which season is suitable to obtain a highest number of actinomyces colonies.

colonies was noticed. This fact was confirmed by the following experiment. 4 g of each of 12 soil samples were divided into two parts and one part was poured with one cc buffer solution at pH 7.0 and the other with the same amount of solution at pH 8.0 and kept at 25° for 30 days. After such treatment each of both soil samples were suspended in 10 cc saline

TABLE V.

The Number of Actinomyces Colonies in relation to the Seasons and to pH of Soils.

Soil sample No.	Spring 10/IV 16°C		Summer 24/VIII 30°C		Autumn 16/X 19°C		Winter 12/I 2°C	
	pH	Number	pH	Number	pH	Number	pH	Number
1	5.7	8	5.6	4	5.8	12	5.6	1
2	5.6	8	5.6	5	5.6	8	5.6	2
3	5.4	10	5.6	7	5.4	9	5.4	2
4	5.6	24	5.4	11	5.5	35	5.5	4
5	5.4	9	5.4	3	5.4	11	5.5	1
6	5.4	7	5.4	4	5.6	6	5.4	1
7	5.6	8	5.5	6	5.6	14	5.6	3
8	5.6	5	5.6	5	5.6	6	5.6	0
9	5.4	3	5.4	2	5.5	5	5.4	4
10	5.4	8	5.4	8	5.4	11	5.4	3
11	5.6	11	5.6	3	5.6	5	5.6	1
12	5.6	5	5.6	3	5.6	8	5.5	1
13	5.6	9	5.6	7	5.4	16	5.5	5
14	5.4	7	5.4	5	5.6	9	5.5	3
15	5.4	3	5.4	4	5.4	7	5.4	0
16	5.6	6	5.5	4	5.6	5	5.4	1
17	5.4	4	5.4	6	5.4	8	5.4	4
18	5.6	11	5.6	9	5.8	18	5.6	5
19	5.4	3	5.4	2	5.4	4	5.4	0
20	5.4	7	5.4	6	5.5	10	5.4	2

The procedures for cultivation was the same mentioned above.

TABLE VI.

The Number of the Actinomyces Colonies in Relation to the pH of Soils.

Soil samples collected from	Sample number	Averaged pH	Averaged number of colonies obtained in one site
Field	76	5.6	6.9
Hill	94	5.5	6.6
Others	18	5.5	3.8

TABLE VII.

The Number of the Actinomyces Colonies in Relation to pH of Soils.

Averaged pH	Averaged number
5.8	6.5
5.6	6.2
5.4	6.0

and cultivated in a similar way mentioned above. The results are shown

in Table VIII. The significant difference of the number of colonies were noticeable between them.

TABLE VIII.

The Number of Actinomyces Colonies Obtained from Soil Samples being Preserved at Different pH.

Soil samples No.	Soils			
	preserved at pH 7.0		preserved at pH 8.0	
	pH	Number of actinomyces	pH	Number of actinomyces
1	5.6	3	6.8	3
2	5.6	4	6.8	2
3	5.8	8	7.0	7
4	5.8	1	6.8	5
5	5.8	3	7.0	6
6	5.6	1	6.8	3
7	5.8	2	6.6	4
8	5.6	1	6.8	2
9	5.6	2	6.6	2
10	5.8	4	7.0	2
11	5.8	2	7.0	6
12	5.8	4	6.8	4
Mean	5.7	2.9	6.8	3.8

The figures indicate the number of colonies per loop of soil suspension, made in a similar manner mentioned above.

The Screening Method of Antagonistic Strains.

In order to prove antogonistic activities of 1223 strains of streptomyces obtained as mentioned above, following 3 methods were used:—

1) All isolated strains were cultured at 24°, for 7 days on modified Vander Brook's semi-solid media, which composed of

Glucose	10 g
Meat-extract	5 g
Peptone	5 g
NaCl	3 g
$(NH_4)_2SO_4$	2.5 g
K_2HPO_4	1 g
$MgSO_4 \cdot 7H_2O$	0.025 g
Agar	2.5 g
Aq	1,000 cc

The culture filtrates were tested against *Sta. aureus* (Terashima strain) as test organisms by streak slant method. The data is shown in Table IX, and by this test 157 (12.8%) antagonistic strains were obtained. As Terashima strain was known to be very susceptible to some antibiotics, it

was used as test organism. By using such organisms, however, it was apt to detect only such a strain that was active against Gram positive bacteria.

Moreover these procedures are very consumable of time and laborious, and it was not able by this procedures to reveal specific entities of antibiotic activities.

TABLE IX.

Antagonistic Strains Obtained by the Streak Slant Method.

	Antibiotic (dilution) unit against *Sta. aureus*								Total of strains
	10	20	40	80	160	320	640	1280	
No. of strains	51	33	20	20	13	13	10	1	157

2) Therefore, 38 out of 157 strains mentioned above which proved to be high potent and showed some characteristic properties in their basic and aerial mycelium were selected and cultured on following 3 media.

(1) Modified Waksman's medium (Surface culture)

Glucose	10 g
Meat extract	20 g
Peptone	10 g
NaCl	3 g
Agar	2.5 g
Aq.	1,000 cc

(2) Modified Le Page's medium (Surface culture)

Starch	15 g
Peptone	10 g
NaCl	5 g
$FeSO_4 \cdot 7H_2O$	0.01 g
$MgSO_4 \cdot 7H_2O$	0.25 g
Agar	2.5 g
Aq.	1,000 cc pH 7.0

(3) Modified Vander Brook's medium (Shaking culture)

Starch	30 g
Peptone	5 g
$(NH_4)_2SO_4$	2.5 g
$MgSO_4 \cdot 7H_2O$	0.25 g
K_2HPO_4	1 g
NaCl	5 g
$CaCO_3$	3.5 g
Aq.	1,000 cc pH 7.0

The former 2 were used for surface and the latter for shaking culture. The activity of their culture filtrates were tested by serial dilution method against *Sta. aureus* (Terashima) and *E. coli* (Kashiwagi). The results are shown in Table X.

It was desired by this experiment to isolate a strain, which will be strongly inhibitory upon both test organisms and moreover will show a dilution unit ratio against *E. coli* and *Sta. aureus* of 1:8 in a similar manner with that of streptomycin. This purpose was not reached by this experiment, because of limited number of strains used and of complexities of procedures.

3) At last, "cross streaks" were used as a "primary screening method." This method was used at first in our laboratory for the screening of antibiotic aspergilli by Ishida, and afterwards it was reported, that Emerson[20] *et al.* also utilized this procedure for the research of the actinomyces antibiotics. Newly isolated 261 strains from 49 soil samples were inoculated on the center of 1 per cent glucose nutrient agar plate in a circle of 2 cm in diameter and incubated for 5 days, later for 2–3 days, at 25°. Three kinds of bacteria i.e. *Sta. aureus*, *B. subtilis* and *E. coli* were streaked as test organisms around the actinomyces colonies. As the result of this experiment 114 active ones were found under 261 strains (43.7%). Thus to detect the antagonistic strains, this method seems to be most sensitive and now, in 1951 it is used continuously as the "primary screening" method. These antagonistic strains were grouped by their antibiotic spectra as shown in Table XI. It was revealed by the result, that there is no strain, which is only active against *E. coli* and this test organism seems to be rather resistent to all isolated strains.

In order to indicate the degrees of the individual inhibitory activities, following marks were given to each of the strains: 3 marks for a complete inhibition against any one of 3 test organisms, 2 marks for intermediate inhibition, 1 mark for a weak inhibition and 0 for no inhibition and all of the strains were divided into 4 groups by summerized marks given to each of them, as shown in Table XII. By this experiment it was revealed that about one half of the streptomyces strains isolated were inhibitory against none of the test organisms, while only few parts of the strains were highly active against all of the organisms.

The Morphological and Biochemical Classification of the Representative Strains.

Under the antibiotic strains obtained by the above method high potent or morphologically specific ones were selected and studied to determinate their species.

According to Krainsky, 7 kinds of media which differ in their C and N sources both, qualitatively and quantitatively and according to Waksman, synthetic-, nutrient-, and starch agar, gelatin medium, glucose broth, synthetic solution, and potato plug were used.

The representative strains were cultured on these 14 kinds of medium

TABLE X.

Antibiotic Activity of 38 Strains

Strain No.	Species	Kinds of media: WAKSMAN pH	WAKSMAN Date of highest potency	WAKSMAN Against *Sta. aureus*	WAKSMAN Against *E coli*	LE PAGE pH	LE PAGE Date of highest potency	LE PAGE Against *Sta. aureus*	LE PAGE Against *E coli*	VANDER BROOK pH	VANDER BROOK Date of highest potency	VANDER BROOK Against *Sta. aureus*	VANDER BROOK Against *E coli*
3	*Str. roseochromogenus*	7.2	7	400*	50	7.2	7	800	50	7.8	5	100	< 50
10	*Str. flavus*	7.6	7	100	<100	7.2	7	800	<100	6.8	4	100	< 50
18	*Str. roseochromogenus*	7.4	7	800	< 50	7.2	9	1600	<100	6.8	5	1600	< 50
20	*Str. roseochromogenus*	7.8	7	400	50	7.8	7	400	50	6.8	5	400	50
23	*Str. roseochromogenus*	7.4	9	200	50	7.6	9	800	50	6.4	5	1600	50
26	*Str. roseochromogenus*	7.2	9	400	50	7.4	9	800	50	6.8	5	1600	50
27	*Str. griseus ?*	6.8	7	< 200	< 50	7.8	7	< 200	< 50	7.0	4	200	< 50
29	*Str. roseochromogenus*	7.8	9	1600	< 50	7.4	11	3200	< 50	7.0	4	1600	200
30	*Str. roseochromogenus*	7.6	7	200	< 50	7.2	7	200	< 50	6.8	4	200	< 50
31	*Str. roseochromogenus ?*	7.8	11	800	< 50	7.6	11	1600	< 50				
33	*Str. roseochromogenus*	7.8	7	800	< 50	6.6	9	400	< 50	6.8	4	400	< 50
36	*Str. roseochromegenus*	7.8	9	1600	100	7.2	11	3200	< 50	6.6	4	1600	< 50
40	*Str. diastaticus*	7.2	7	100	<100	7.6	7	100	<100				
50	*Str. albosporeus or Str. bobili*	8.6	7	< 200	< 50	8.2	7	< 200	< 50	6.6	5	200	< 50
58	*Str. roseochromogenus*	7.8	7	1600	< 50	7.4	7	800	< 50	6.8	4	1600	200
60	*Str. erythrochromogenus*	7.6	7	200	<100	7.2	7	100	< 50				
65	*Str. ruber*	7.8	7	100	< 50	8.0	7	800	<100				
68	*Str. roseochromogenus*	7.8	9	1600	<100	7.6	9	1600	< 50	7.8	6	3200	100
71	*Str. roseochromogenus*	7.8	9	< 100	<100	7.8	9	200	<100	6.8	4	3200	100
75	*Str. flavus*	7.6	7	< 200	< 50	7.4	7	< 200	< 50	7.0	5	400	< 50

| | | |

TABLE XI.

Grouping of 114 Strains by Their Antibiotic Spectrum.

Active against	No. of strains	%
Sta. aureus, *B. subitilis* and *E. coli*	33	29.0
Sta. aureus and *B. subtilis*	35	30.7
Sta. aureus and *E. coli*	12	10.5
B. subtilis and E. coli	0	0
Sta. aureus only	22	19.3
B. subtilis only	12	10.5
E. coli only	0	0
	114	100.0%

TABLE XII.

Grouping of 114 Strains by Their Antagonistic Activities.

Marks	Number of strains	%	
0	147	56.3%	
1–3	57	21.9%	
4–6	47	17.2%	43.7%
7–9	12	4.6%	
	261	100.0%	

ed from 188 places were cultivated in several kinds of fluid culture media and the culture filtrates were tested for their inhibitory activities, using mainly *Sta. aureus* (Terashima strain) as test organisms, and 157 (13%) strains were found to be antagonistic. The above method is well reliable in finding inhibitory strains whose activities were detectable in their culture filtrates. It was not so sensitive, however, to reach a highest detection rate of effective strains.

3) By the so-called "Cross streak method" 144 (44%) active strains were selected from 261 actinomyces. This method is very simple and sensitive to find many antibiotic strains and to examine a specific "antibacterial spectrum." The development of this task will be reported in the next report.

4) The representative strains were classified, according to Krainsky and Waksman, into several species by the cultural properties on several kinds of media. Although a difinite identification of all strains was scarcely possible, many strains were found to belong to *Str. roseochromogenus*.

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Some Remarks on Measurements of Electrical Excitability of the Human Eye.

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Introduction.

Electrical stimulation of the eye is becoming a favorite means for researches of vision. Bourguignon,[1] Iwama[2] and others measured the chronaxie of the human eye with electrically evoked phosphenes as index. Kravkov and Galochkina[3] found that the sensitivity of the human eye to spectral lights is either enhanced or depressed during electric polarization, and the same relation was confirmed by Gernandt[4] in cat's eye. Granit[5] showed that the sensitivity of cat's eye to colored lights is enhanced by polarization in three preferential regions of the spectrum. It is evident from these experiments that electrical stimulation provides a useful means for analyzing visual function. It is, however, a great disadvantage of the method of polarization hitherto employed that it is difficult to stimulate selectively any part of the retina we wish to study, because the distribution of currents within the eye is generally too diffuse and difficult to be controlled adequately from without. However, Motokawa's discovery of the electrical supernormal phase following a brief illumination has made it possible to confine electrical stimulation to any desired region of the retina by sensitizing it with pre-illumination and then by applying a threshold electric stimulus.

Determination of electric thresholds is certainly not so easy in the human eye as in nerve-muscle preparations, but under controlled experimental conditions and with due precautions it is not difficult to work with the same degree of accuracy in both cases. In the present paper, factors affecting accuracy in the measurements of electrical excitability of the human eye will be pointed out and the way to avoid these difficulties will be described.

Experimental.

Method and Apparatus.

A single constant current pulse of 100 msec. in duration is applied

to the human eye through a pair of silver electrodes of 2×1.5 cm² in size, placed one on the forehead slightly above the eyebrow and the other on the homolateral temple of the subject. The contact of the electrodes with skin is secured by the use of electrode paste.

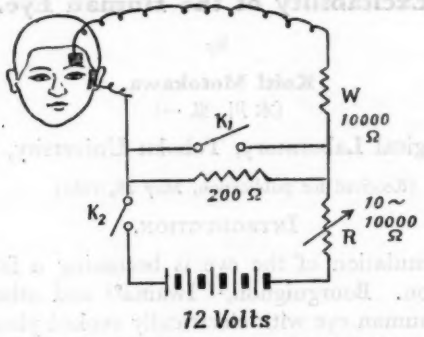

Fig. 1. Circuit used for electrical stimulation of the eye.

The circuit used for electrical stimulation is shown in Fig. 1. The contacts K_1 and K_2 are opened in succession by means of a spring rheotome at a constant interval of 100 msec. The duration of 100 msec. is about 2 times the utilization time for the rheobase of the eye. The use of a current of too long duration should be avoided, because there appear phosphenes at the closing and the opening of the current. A too brief shock should also be avoided, because it stimulates selectively only retinal elements with a small time constant. Alternating currents act also selectively on retinal elements according to their frequency.

The strength of the stimulating current is controlled by variable resistance R in the figure. A constant resistance W of 10,000 ohms inserted in series in the stimulating circuit serves to reduce the effect of variation in resistance of the tissue.

We usually place a cathode on the forehead, but little alterations in quality of results obtained with an anode on the forehead were found. The value of ζ determined with the cathode on the forehead was found greater only by 2 or 3 than that determined with the anode on the forehead under otherwise one and the same experimental conditions.

The color and location of phosphenes are not taken into account, because they can hardly be distinguished when the intensity of the electric stimulus approaches the threshold.

Factors Affecting Electrical Thresholds of the Human Eye.

(1) Adaptation state. The electrical threshold is found lowest

several minutes after entering a dark room, then rises rather rapidly and attains a fairly constant level in about 20 minutes. It takes, however, about 40 minutes or so to reach a steady state in a strict sense. We usually commence experiments after preliminary dark adaptation of about 20 minutes.

(2) Resistance of skin. The absolute value of threshold depends greatly upon the electrical resistance of the skin. Sweating lowers the resistance and in consequence electrical thresholds. In order to keep the threshold value for the resting level constant throughout an experiment, it is absolutely necessary to maintain the room temperature and ventilation constant.

(3) Attention and attitude of the subject. Weak phosphenes caused by electric stimuli near the threshold can be perceived only when the subject concentrates his attention to them. It is sometimes difficult for a beginner to concentrate attention to a phosphene appearing in the periphery of the retina. Electrical phosphenes are sometimes very similar in appearance to intrinsic light of the retina, but have a characteristic that they appear in definite relation to the electric stimulus, while intrinsic light appears at random.

In order that the subject might discriminate the weakest electric phosphene from the background of intrinsic light, he must be informed of the moment at which the electric stimulus is given, and for this purpose,we give at least two anticipatory signals; the one is a verbal signal which is given a few second prior to the electric stimulus and the other a click delivered 0.5 sec. prior to it. Such a short interval between the click and the electric stimulus is advantageous for identification of an electric phosphene. When the click and the electric stimulus are given at the same time the discrimination of the electric phosphene from intrinsic light is rather more difficult.

(4) Fatigue. Electric phosphenes are diminished in intensity by fatigue, and the threshold determined with an electric phosphene as the index under well controlled conditions may be used as a sensitive measure for fatigue. The method of electric flicker for the study of fatigue is based on this principle (Motokawa and Suzuki[8]). The following experience also serves to show a close connection of the electric threshold of the eye with fatigue. One of our subjects engaged in researches on color vision used to show an electric threshold by about 50% higher than ususal after a few day's journey, and the value gradually returned to the usual level in several days of routine laboratory work. It is, however, to be noted that, though the electric threshold is thus very sensitive to fatigue, the effect of illumination as measured by ζ is far less sensitive to fatigue. As will be shown later, the value of ζ depends not so much upon the absolute

value of electric thresholds as upon the relative value. Therefore, Motokawa's method for color vision researches is applicable even in a slightly fatigued state of the subject.

The Method for Determining Electric Thresholds of the Eye.

There are two kinds of threshold, namely that for appearance of response and that for disappearance, and the average of the two is usually taken as the threshold value. However, measurements of any one of them will do when only relative values are needed as in determination of ζ.

So far as the electric threshold of the human eye is concerned, the threshold for disappearance is preferable, because it can be determined more accurately. The procedure for determining a threshold for disappearance is as follows: Starting with a voltage of 2~3 volts which is sufficient to cause a distinct phosphene, the stimulating voltage is lowered step by step in such a manner as is exemplified in Table I. When the voltage is so much reduced that the subject feels it difficult to discriminate an electric phosphene from the background of intrinsic light, he demands a comparing procedure. The procedure consists of delivering in succession at an interval of several seconds two electric stimuli, one of which is the stimulus in question and the other one far below the threshold. The subject is requested to answer the question which the stronger stimulus is, and informed every time whether his answer is right or wrong.

The same comparing procedure is repeated with stimulating voltages reduced step by step until the subject can no longer distinguish the two stimuli. Near the threshold the voltage is graded by about 1%, because a trained subject can discriminate a difference of about 1%. If the answer given by the subject is wrong, the test with the same voltage is repeated three times, and it is decided that he can no longer discriminate when he fails twice. In these tests the subject is allowed to request a further trial when he thinks that his failure is due to some accidental disturbance or when he failed in judgement though a fairly distinct difference was perceived between the effects of the two stimuli.

Excitability Curves and Multiple Thresholds.

As has been disclosed by Motokawa,[6] the electrical excitability of the eye recovers rapidly following a brief illumination and becomes supernormal. Such supernormality may be used as a measure of the physiological effect caused by the illumination, and we express the degree of supernormality with a quantity ζ which is defined by the relation: $\zeta=100\ (E-E_o)/E_o$, where E and E_o represent respectively the electrical excitability at the moment in question and its resting level in the dark-adapted state. ζ is a function of time elapsed since termination of a light-stimulus. Some examples of ζ-t curves or excitability curves are shown in Fig. 2. The

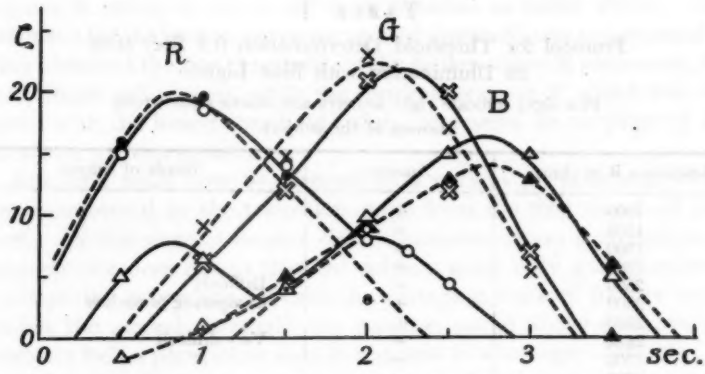

Fig. 2. Excitability curves. Curves R, G and B were obtained after 2 seconds exposure to red (650 mμ), green (530 mμ) and blue (465 mμ) lights respectively. Broken and continuous curves were obtained at different sittings.

data represented by broken lines were obtained at a sitting and those expressed by continuous lines at another. As can be seen in the figure, the two series of experiment show in general satisfactory agreement with each other except in certain limited portions of the curves. There is a fairly striking discrepancy between the initial parts of the broken and continuous B curves (curves marked by triangles). This difference is too great to be regarded as due to the error of measurements. As a matter of fact, this difference is due to the circumstance that in the one case the threshold of red receptors involved was not measured while both thresholds for red and blue receptors were measured in the other case. (note 2 threshold values for 0.5 and 1 sec.)

As shown in Table I, two kinds of thresholds were obtained at the moment 0.5 sec. after the illumination with blue light. As the resistance R was increased (as the voltage was decreased), at a certain voltage the subject found it almost impossible to discriminate an electric phosphene from the background of intrinsic light (the first threshold). On increasing the resistance further, discrimination became easier and finally the second threshold was reached. The first threshold determined in this way refers to the blue process, and the second to the red process, for the ζ-value corresponding to the first threshold belongs to the ascending branch of the curve having its maximum at about three seconds, while the ζ-value corresponding to the second threshold falls on the R curve with its maximum at about one seconds. The appearance of the double threshold is quite natural, because blue light stimulates not only the blue receptor but also the red receptor though to a lesser degree. When green light is used for pre-illumination it is possible to find three thresholds, because green light

TABLE I.

Protocol for Threshold Determination 0.5 Sec. after an Illumination with Blue Light.

Plus signs indicate right answers and minus signs wrong answers of the subject.

Resistance R in ohms	Answer	Words of subject
1000	+	
1500	+	
1900	+	
2300	+	
2500	+	Difficult
2600	+	Comparing procedure
2650	+	
2680	+	Very difficult
2700	−	
2700	−	
2700	+	It became easier
2740	+	
2780	+	
2780	+	
2810	+	
2840	+	
2840	+	
2840	+	
2870	+	
2870	+	
2870	+	
2900	−	Almost impossible
2900	+	
2900	−	

stimulates red, green and blue receptors to a considerable degree. However, we do not always encounter multiple thresholds, for they can be obtained only in special experiments with sufficiently fine gradation of stimulating voltages; when gradation is not small enough, higher thresholds are overseen and only the lowest threshold is obtained.

We usually construct an excitability curve with such lowest thresholds, so that it represents an envelope of excitability curves for two or three kinds of receptors involved. When the stimulating voltage is graded in sufficiently small steps a higher threshold is encountered, but the lower ones can be neglected by an experimenter not aware of the possibility of multiple thresholds. Being unaware of this situation, one may be disappointed at such a great discrepancy between the threshold values determined under one and the same experimental conditions. Especially when the procedure for threshold determination is not systematic, a kind of threshold is encountered on one occasion and another on another occasion. In consequence the result seems exceedingly variable, and one may be led to the misunderstanding that no quantitative study would be possible with such variable data.

The discrepancy found between the later portions of the broken and

continous R curves is due to the same situation as stated above. In the experiment for the broken curve the second threshold was not pursued after having obtained the first threshold. The broken curve R represents, therefore, a simple red process, while the continuous curve R which was constructed with the lowest threshold values, represents an envelope of a red process and a green process.

Attention must now be directed to a curious phenomenon which is often encountered in the transition stage from the first threshold to the second. In this stage, a weaker electric stimulus causes a phosphene, but a stronger one does not so that the subject gives only wrong answers in the comparing procedure. When the voltage is reduced further and approaches the second threshold the paradox stated above disappears and the subject feels a phosphene only in response to a stronger stimulus. This phenomenon makes us foresee the existence of the second threshold, although its physiological mechanism is not clear.

It is a usual manner that stimuli above and below the threshold are given alternately in order to approach the threshold from both sides, but this method for determining thresholds cannot be applied to the eye in view of the existence of multiple thresholds and of the paradoxical phenomenon stated above. It is to be noted that multiple thresholds can be determined more easily in color-blind persons than in normal subjects.

Summary.

Measurement of the electrical excitability of the eye provides a useful means for analyzing visual function. Determination of electric thresholds of the eye necessitates, however, the following special precautions.

(1) A single constant current pulse of 0.1 sec. in duration is adequate as an electric stimulus, because it can stimulate uniformly all kinds of retinal elements. On the contrary, a brief condenser discharge and alternating currents of definite frequency stimulate selectively a certain kind of element.

(2) A weak phosphene caused by an electric stimulus near the threshold can hardly be distinguished from intrinsic light of the retina unless the effect of the stimulus in question is compared with that of a stimulus far below the threshold.

(3) The threshold for disappearance of phosphenes can be determined more accurately than that for appearance.

(4) Retinal elements of different kind show different thresholds, and this is the most important thing to be borne in mind in determination of the electrical excitability of the eye following illumination.

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The Cardio-Accelerator Fibers in the Vagus Nerve.

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Although some investigators claim the existence of the cardioaccelerator fibers which emerge from the medulla oblongata through the vagus nerve, this problem does not seem to be clearly decided.

Rutherford (1869)[1], Schiff (1879)[2] and Tulgan (1923)[3] observed an increase in heart rate on electrical stimulation of the peripheral end of the cut cervical vagus after paralysis of the cardio-inhibitory endings with atropine.

On the other hand, Boehm (1875)[4], Rossbach (1875)[5] and Hering (1924)[6] denied that stimulation of the vagus nerve after atropine could accelerate the heart rate. Then Jourdan and Nowak (1936)[7] demonstrated the acceleration of the heart on electrical stimulation of the peripheral end of the cut vagus rootlets intracranially after application of atropine in the dogs, and Kabat (1939)[8] demonstrated the vagal caridoaccelerator fibers on electrical stimulation of the cervical vago-sympathetic trunk and of the vagus rootlets intracranially in the dogs after atropine. More recently Pannier (1946)[9] found vagal cardio-accelerator fibers in 80 per cent of the dogs.

These experiments besides Pannier's, of which the details were not clear, are undertaken by using atropine in order to eliminate the cardioinhibitory action.

Okinaka and his collaborators (1942)[10] confirmed that the cardioinhibitory fibers of the vagus nerve of the dog were localized in the caudal portion of the myelencephalic rootlets of the accessory nerve (in Fig. 1, AH and the caudal part of AM). If the probable vagal accelerator fibers run through the other portion of the rootlets, they will be demonstrated by eliminating the cardio-inhibitory action by cutting the portion of the rootlets which contain the cardio-inhibitory fibers without the use of atropine. This assumption was considered and following experiments were undertaken.

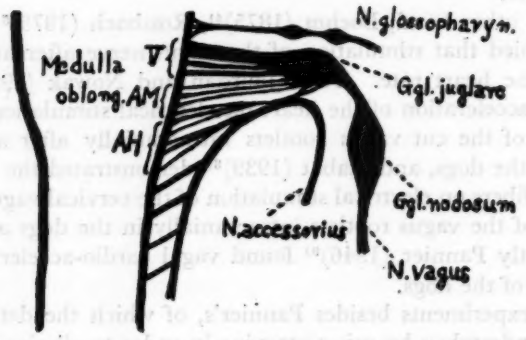
Experimental.

Nineteen dogs were used. Most of them were anesthetized with morphine hydrochloride which was injected subcutaneously in dosis of 6–8 mg per kg. of body weight 30–60 minutes before the stimulation. Blood pressure and heart rate were recorded from the femoral artery by means of a mercury manometer on a smoked kymograph drum. The Du Bois-Reymond induction coil was used to provide electrical stimulation. The duration of stimulation were 10–20 seconds. In 7 cases electrocardiographs were recorded from the right upper limb and the left lower limb at the same time. The experiments were carried out in the following manner.

A) *Electrical stimulation of the vagus rootlets V (see Fig. 1) intracranially.*

Right vagus rootlets were cut at the junction of the medulla oblongata and the peripheral end of the cut rootlets V was stimulated. Four dogs were used. In one case, because of bleeding, stimulation was impossible. And three cases were successfully stimulated, but the cardiac acceleration

Fig. 1



was not demonstrated in any case. On the other hand bradycardia was often observed presumably by the spread of stimulation to rootlets AM and AH.

B) *Electrical stimulation of the vagus rootlets V, two weeks after the section of rootlets AM and AH, which contain the cardioinhibitory fibers.*

In order to eliminate the cardio-inhibitory action, rootlets AM and AH were cut intracranially and two weeks later, when the cardioinhibitory fibers degenerated, the vagus rootlets V was cut and the peripheral end was stimulated intracranially. Two dogs were used. One died on the

first post-operative day and in the other slight cardiac acceleration was demonstrated by stimulation.

(The following figures indicate numbers of heart beats in periods of 5 seconds).

11.0 9.5 12.0 12.0 14.0 14.0 13.0 11.0
stimulation
(10 sec.)

In this experiment cardiac inhibition was not observed because of the degeneration of the cardio-inhibitory fibers. But the stimulation of the rootlets V two weeks later intracranially is difficult because of bleeding from the granulation tissue which developed from the previous operation. Consequently other experiments were undertaken.

C) *Electrical stimulation of the cervical vago-sympathetic trunk two weeks after the section of rootlets AM and AH which contain the cardio-inhibitory fibers.*

As in the previous experiments, rootlets AM and AH were cut intracranially and two weeks later, when the cardio-inhibitory fibers degenerated, the cervical vago-sympathetic trunk was cut at the level about 3 cm peripheral from the nodose ganglion. And the peripheral part was stimulated near the cut end. Five dogs were used. In three of them moderate cardiac acceleration was observed as follows.

(The following figures indicate numbers of heart beats in periods of 5 seconds).

16.0 16.0 16.0 17.0 19.0 18.0 16.0 16.0
stimulation
(10 sec.)

4.0 4.5 5.0 4.5 4.5 6.5 6.5 5.0 4.5
stimulation
(10 sec.)

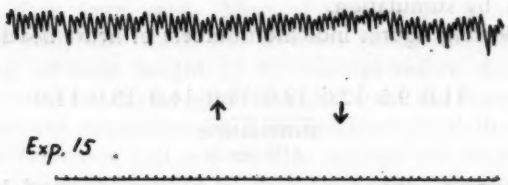
8.0 8.0 8.0 8.0 8.5 9.5 9.5 8.5 8.5 9.5 8.0
stimulation
(15 sec.)

Fig. 2 and Fig. 3 also show graphically results of these experiments. In one of the remaining two, cardiac acceleration was observed by stimulation after the extirpation of the right stellate ganglion (Fig. 4).

D) *Electrical stimulation of the cervical vago-simpathetic trunk two weeks after the section of rootlets AM and AH on the right side and the extirpation of the right stellate ganglion.*

In order to heighten the tonus of the vagal cardio-accelerators, and also to avoid possible stimulation of cardio-accelerator fibers from the

Fig. 2



Beginning and end of the stimulation are marked with an arrow. Time in seconds.

Fig. 3

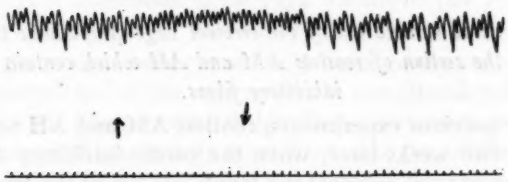
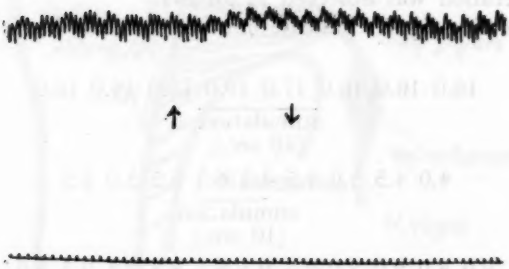


Fig. 4



spinal cord, the right stellate ganglion was extirpated together with section of the cardio-inhibitory rootlets. Two weeks later the cervical vago-sympathetic trunk was cut and stimulated at the same level as in the previous experiments. Eight dogs were used. One died on the first post-operative day and stimulation was performed in seven dogs. Cardiac acceleration was demonstrated in three. In one of these, the acceleration was especially marked, before stimulation 4.5 beats per 5 seconds, after stimulation 10 per 5 seconds (Fig. 5).

Fig. 6 and Fig. 7 also show the results in this experiment. In two of the remaining four, cardiac acceleration was observed only after the in-

Fig. 5

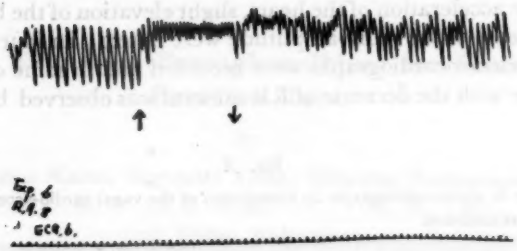


Fig. 6

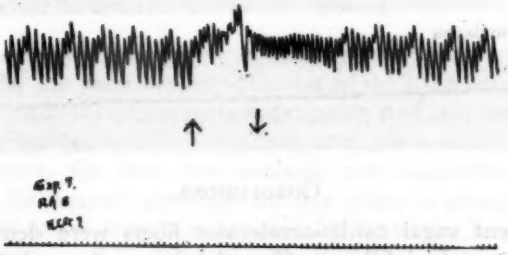
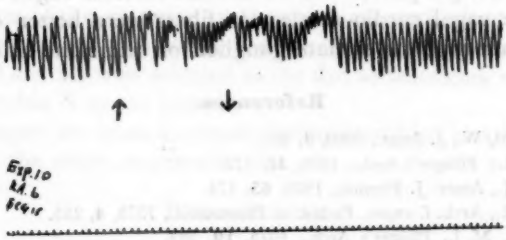


Fig. 7



jection of atropine. In these cases the existence of rootlets AM was revealed in the autopsy which was made after the experiment. Therefore cardiac acceleration occurring only after atropine seems to be due to the remaining cardio-inhibitory fibers in the rootlets AM.

In these 10 experiments in which the vagal cardio-accelerator fibers were demonstrated, the stronger the stimulation, the clearer was the acceleration, and some latent period was observed between the onset of stimulation and the appearance of its effect. The effect was most marked 5 to 10 seconds after the onset of the stimulation, and it continued from 5

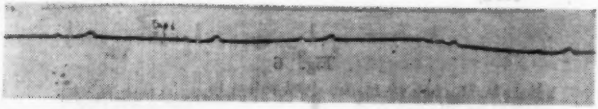
to 30 seconds after the end of the stimulation.

With the acceleration of the heart, slight elevation of the blood pressure and the decrease of the pulse-amplitude were noted. In four cases of these experiments electrocardiographs were recorded and in some cases lowering of the T wave with the decrease of RR interval was observed by stimulation (Fig. 8).

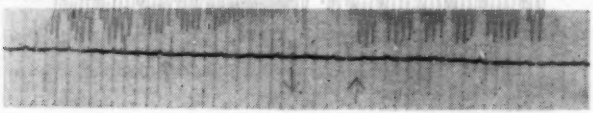
Fig. 8

Change in electrocardiograph by stimulation of the vagal cardio-accelerator fibers

Before stimulation



After stimulation



Conclusions.

1. Efferent vagal cardio-accelerator fibers were demonstrated by section of the cardio-inhibitory fibers in the myelencephalic rootlets of the accessory nerve followed by stimulation of the cut myelencephalic vagus rootlets or peripheral end of the cut cervical vagus nerve.

2. These vagal cardio-accelerator fibers were better demonstrated by the extirpation of the stellate ganglion on the same side.

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A New Dye (Bromphenolblue) for Liver Function Test.

By

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The dye for the excretory function test of the liver has been investigated by many authors and its functional capacity had been tested clinically by excretion of the dye into the feces, bile, urine and retention in the blood stream. However, the first two methods are unsuitable for clinical purposes. As Nakagawa[1] reported, the dye which is eliminated only in the bile and not in the urine is most advisable for testing by the retention in the blood stream, while, for testing by the urine, it is preferable that the greater part of the dye appears in the bile and only a small proportion in the urine in normal condition, and that an increased output and a prolonged duration of the excretion in the urine are noticed when a disturbance of the eliminating function occurs. Bromsulphalein, rose bengal and congo red etc. are now selected as the dye as belonging to the former, and only azorubin S to the latter.

In this paper we want to report bromphenolblue can be applied to the testing by the urine as azorubin S.

Experimental.

Preliminary Experiment.

First of all, the elimination of the dye in the urine and bile was investigated in a dog. The urine is taken by a catheter and the method which was introduced by McMaster and Elman[2] and which is most ideal for the purpose of the collection of bile is employed. 3.0 c.c. of 0.5 or 1.0% soultion is injected into the large saphenous vein of a dog and collections are performed every 30 minutes till the disappearance of the dye. The percentage readings of excretion for injected amount are estimated.

The percentage eliminated in the bile reached its maximum in ½ to 1 hour and indicated 79.3% in 0.5% solution, 76.4% in 1.0% solution

during 5 hours, showing 84.3, 85.8% in total respectively.

The total excretion in the urine was 7.4 and 5.1% in 0.5 and 1.0% solution respectively. The time required for first appearance of the dye in the urine was 9 minutes in each solution.

Moreover, we performed the same experiments using 1.0% azorubin S solution. The excretion in the bile was 72.3% in 5 hours and 74.3% in total. The time required for first appearance in the urine was 5 minutes and toal excretion showed 12.4%.

After all, the greater part of these two dyes is similarly eliminated from the liver and only a very small proportion from the kindney. Moreover, we found more increased output of bromphenolblue than that of azorubin S in the bile and contrary result in the urine, showing that the total output of bromphenolblue in both bile and urine is more than that of the other.

On the other hand, it is confirmed by the fact that bromphenolblue never appears in the gastrointestinal juice. Even a trace of the colour could not be found in the gastric and intestinal content by post-mortem examination one hour after the injection into the blood stream of a dog whose common duct was tied off by a ligation. Furthermore, in fractional collection of the gastric juice in men for 2 hours by means of Rehfuss tube after giving it intravenously, no colour of the dye was detected.

Concerning the toxicity of the dye, it was found that the lethal dose for a frog was 1.5 mgrms. per 1 grm. of body weight in bromphenolblue, 2.5 mgrms. in azorubin S. However, the administrated amount of bromphenolblue less than that of azorubin S will bring about the similar effect on the human body.

Moreover, we observed the elimination in the urine before and after experimental disturbance of the liver in a dog. The total excretion after giving 3.0 c.c. of 0.5% solution intravenously showed 8.8 and 8.5% before the damage. While, it increased remarkably showing 35.4% 1 day, 54.1% 3 days after giving 1.0 c.c. of chloroform per kgrm. subcutaneously. 12 days after injection, it decreased to 8.1%.

From these facts above mentioned, it is concluded that this dye can be applied to clinical purposes.

Clinical Experiment.

Method. 4.0 c.c. of 0.5% solution is administered intravenously and the urine is taken hourly during 5 hours, and later every 2 hours till the disappearance of the dye in the urine. Each specimen is measured and the amount of dye excreted is estimated colorimetrically. The percentage of excretion for injected amount is obtained.

Results. In 7 normal persons, the excretion was all below 12.0% and

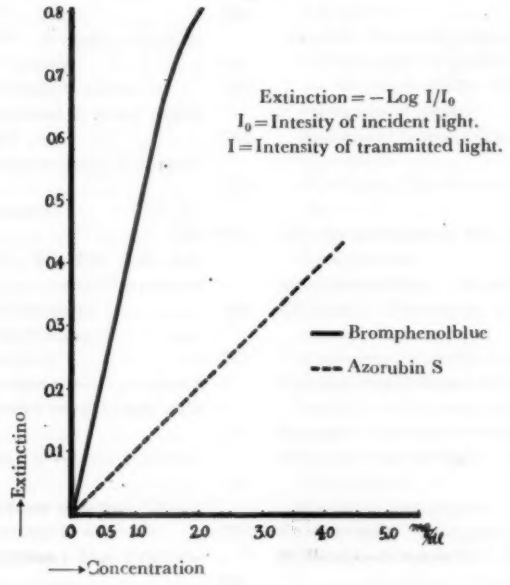
the time required for disappearance was less than 5 hours. On the other hand, 10 out of 12 cases of liver and gall-bladder diseases indicated the excretion over normal reading, especially 3 cases of hepatitis and 1 case of gall stone showed a marked increased excretion of over 50% indicating a parallel result with that of azorubin S.

Remarks.

Bromphenolblue belongs to the phthalein group of indicater. It is a yellow powder and presents a brilliant violet colour above pH 4.6. Its solution is obtained by adding 1.5 c.c. of 0.1 *N* NaOH per 100 mgrms., because it is slightly soluble in water.

The following fact shows that we can distinguish more exactly the difference of concentration in bromphenolblue as compared with azorubin S by photoelectric colorimeter. As is shown in Fig. 1 in which the concentration of the dye solution in abscissa and extinction in ordinate are indicated, the curve of bromphenolblue solution is steeper than azorubin S.

Fig. 1



Conclusions.

1. Bromphenolblue appears in the bile in a very high concentration

and in the urine in a small proportion. Its elimination in the urine increases remarkably in the liver disturbance.

2. This dye is one of the most suitable for the liver function test estimated by means of the excretion in the urine.

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